Evolutionary Origin and Maintenance of Sociality in the Small Carpenter Bees

by

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THESIS SUMMARY

Many arthropods exhibit behaviours precursory to social life, including adult longevity, parental care, nest loyalty and mutual tolerance, yet there are few examples of social behaviour in this phylum. The small carpenter bees, genus *Ceratina,* provide important insights into the early stages of sociality. I described the biology and social behaviour of five facultatively social species which exhibit all of the preadaptations for successful group living, yet present ecological and behavioural characteristics that seemingly disfavour frequent colony formation. These species are socially polymorphic with both solitary and social nests collected in sympatry. Social colonies consist of two adult females, one contributing both foraging and reproductive effort and the second which remains at the nest as a passive guard. Cooperative nesting provides no overt reproductive benefits over solitary nesting, although brood survival tends to be greater in social colonies.

Three main theories explain cooperation among conspecifics: mutual benefit, kin selection and manipulation. Lifetime reproductive success calculations revealed that mutual benefit does not explain social behaviour in this group as social colonies have lower per capita life time reproductive success than solitary nests. Genetic pedigrees constructed from allozyme data indicate that kin selection might contribute to the maintenance of social nesting as social colonies consist of full sisters and thus some indirect fitness benefits are inherently bestowed on subordinate females as a result of remaining to help their dominant sister. These data suggest that the origin of sociality in ceratinines has principal costs and the great ecological success of highly eusocial lineages occurred well after social origins.

Ecological constraints such as resource limitation, unfavourable weather conditions and parasite pressure have long been considered some of the most important selective pressures for the evolution of sociality. I assessed the fitness consequences of these three

ecological factors for reproductive success of solitary and social colonies and found that nest sites were not limiting, and the frequency of social nesting was consistent across brood rearing seasons. Local weather varied between seasons but was not correlated with reproductive success. Severe parasitism resulted in low reproductive success and total nest failure in solitary nests. Social colonies had higher reproductive success and were never extirpated by parasites. I suggest that social nesting represents a form of bet-hedging. The high frequency of solitary nests suggests that this is the optimal strategy when parasite pressure is low. However, social colonies have a selective advantage over solitary nesting females during periods of extreme parasite pressure.

Finally, the small carpenter bees are recorded from all continents except Antarctica. I constructed the first molecular phylogeny of ceratinine bees based on four gene regions of selected species covering representatives from all continents and ecological regions. Maximum parsimony and Bayesian Inference tree topology and fossil dating support an African origin followed by an Old World invasion and New World radiation. All known Old World ceratinines form social colonies while New World species are largely solitary; thus geography and phylogenetic inertia are likely predictors of social evolution in this genus.

This integrative approach not only describes the behaviour of several previously unknown or little-known *Ceratina* species, but highlights the fact that this is an important, though previously unrecognized, model for studying evolutionary transitions from solitary to social behaviour.

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OVERVIEW OF THESIS CONTENTS

Chapters following the general introduction are written in manuscript format and have either been published (Chapters 2, 3, 5 and 6), or will be submitted with minor modifications (Chapter 4). References for Chapter 1 (introduction) and Chapter 7 (general discussion) are combined and presented at the end of Chapter 7.

Chapter 2 has been published in *Insectes Sociaux* (vol 57, pp 403-412). This manuscript is co-authored with Miriam Richards and Michael Schwarz who supervised the project and gave statistical and editorial advice.

Chapter 3 has been published in the *Biological Journal of the Linnean Society* (vol 103, pp 57-67). This manuscript is also co-authored with my supervisors Miriam Richards and Michael Schwarz.

Chapter 4 is co-authored with Mark Adams who helped with allozyme electrophoretic work, Miriam Richards and Michael Schwarz who supervised and gave editorial advice.

Chapter 5 has been published in the *Journal of the Kansas Entomological Society* (vol 82, pp 194-209). This manuscript is co-authored with Miriam Richards who gave statistical and editorial advice, and Michael Schwarz who assisted with field work and also provided statistical and editorial advice.

Chapter 6 has been published by *Molecular Phylogenetics and Evolution* (vol 55, pp 1042-1054). This manuscript is co-authored with Tom Chapman and Andrew Craigie who provided technical support, Miriam Richards who supervised, Steve Cooper who provided expertise for phylogenetic analyses, and Michael Schwarz who supervised, assisted with field work and provided statistical and editorial guidance.

Chapter 7 is a review paper on the Ceratinini combining literature review with findings from this thesis to propose a mechanism for the evolution of sociality in the small carpenter bees.

Chapter 1: General Introduction

This thesis is about the social evolution of the small carpenter bees. Small carpenter bees have long been considered solitary (Wilson 1971; Michener 1974; Michener 2000), but increasing evidence reveals that many species exhibit behaviours unusual to solitary insects while some species are social (Sakagami and Maeta 1977; Daly 1988; Rehan et al. 2009). My research on the origins and maintenance of sociality uses a two-fold approach. The first approach requires the construction of molecular phylogenies to compare and contrast the age and frequency of social behaviour in primitively eusocial bees. The second approach entails the study of socially polymorphic species to compare and contrast the fitness consequences and ecological determinants of group living.

Part 1: Phylogenetic Contrasts and the Origins of Sociality

Sociality has arisen most frequently and with greatest complexity within the social Hymenoptera: ants, bees and wasps, over 65 million years ago (Brady et al. 2006). The highly eusocial bees are found in the family Apidae, subfamily Apinae, tribes Apini and Meliponini. Both are obligately eusocial with no reversion to single generation, cooperative colonies (parasocial), or solitary life (Michener 2000). It has been argued that such strong specialization introduces an evolutionary 'point of no return' and that highly social species experience different selective pressures than those undergoing social transitions (Wilson and Hölldobler 2005).

Understanding the transition to sociality requires a group of closely related taxa exhibiting broad social, taxonomic and geographic diversity. Socially polymorphic lineages (those with both solitary and social species) retain the plasticity to illuminate the evolutionary steps from solitary to social life. A prime candidate is the Xylocopinae, sister subfamily to

the Apinae. The Xylocopinae provide numerous contrasts to offer insights into the origin of sociality with their range of solitary to social forms. Morphological and behavioural observations in combination with modern molecular phylogenetics provide independent data sets to assess the origin and evolution of these taxa.

Age and Phylogenetic Relationships in the Xylocopinae

Within the long tongued bees are two families, Megachilidae and Apidae. The family Megachilidae are an assemblage of solitary leafcutter bees and the family Apidae contain a variety of socially polymorphic tribes ranging from solitary to éusocial. Within the family Apidae, Xylocopinae are the sister subfamily to Apinae (Cardinal et al. 2010).

The Xylocopinae are divided into four tribes of xylophilous bees, namely Manueliini, Xylocopini, Allodapini and Ceratinini. Morphological phylogenetics suggests that Manueliini is the basal tribe of Xylocopinae after which Xylocopini followed by Ceratinini and Allodapini evolved (Roig-Alsina and Michener 1993). Allodapini and Ceratinini have long been thought of as sister tribes due to their morphological similarities and the discovery of 40 million year old fossils, the Boreoallopadini, discovered in Baltic amber (Engel 2001). Recent molecular studies of the Xylcopinae support previous morphological hypotheses on the phylogenetic relationship among the tribes (Flores-Prado et al. 2010).

Independent assessment of the origin and age of each tribe has proven informative. However, many of these findings contradict behavioural and morphological hypotheses. The relict tribe Manueliini is found exclusively in Chile and Argentina and much remains unknown about their biology and evolution. The age and origin of this tribe has never been examined and behavioural data are scarce. The lack of study in this tribe is likely due to its lack of diversity, consisting of a lone genus and only 3 species. Also, their narrow

geographic range leaves little room for contrast among the 3 sympatric species (Daly et al. 1987).

The large carpenter bees, Xylocopini, are found on all continents. All species belong to a single genus (*Xylocopa*) with 450 described species. The subgeneric ranks are still in dispute, with 33 or 51 morphological groupings described (Minckley 1998; Hurd and Moure 1963). Recent molecular phylogenetic work has provided a second independent assessment of the Xylocopini in which robust sampling across the tribe has suggested an Asian origin approximately 45 million years ago (Leys et al. 2002).

The Allodapines, tribe Allodapini, have a narrow distribution limited to the old world tropical and austral regions, southern Eurasia and sub-Saharan Africa (Michener 1977). This tribe consists of 12 described genera and hundreds of species. Recent molecular phylogenetics has verified the monophyly of each genus (Chenoweth et al. 2007). *Macrogalea,* found in sub-Saharan Africa and Madagascar, is the basal genus of the tribe (Schwarz et al. 2003). Molecular clock and fossil calibration has situated the origin of the tribe in eastern Africa 47 million years ago (Chenoweth et al. 2007).

The small carpenter bees, tribe Ceratinini, have a cosmopolitan distribution. Systematics of this tribe are under revision with new subgenera and species described annually. In 2000, Terzo produced the first complete phylogeny of the tribe assessing subgeneric relationships. Ceratinini comprise one genus with 23+ subgenera and hundreds of species descriptions. Terzo (2000) was unable to determine the most ancestral subgenus due to a basal polytomy and a lack of outgrouping. The age of the ceratinines remains unknown but morphological phylogenetics of the subfamily imply that Ceratinini is the sister tribe to the Allodapini (Sakagami and Michener 1987).

Social Behaviour of the Xylocopinae

The Xylocopinae are unusual among bees due to numerous synapomorphic, subsocial traits including adult longevity, extended maternal care, trophallaxis, mutual tolerance, and shared hibernacula (Michener 1990b).

Manueliini are small slender bees that nest in dead stems or decomposing wood. Nidification entails boring an entrance through stems or timber against the grain and forming T-shaped branching tunnels within the wood along the grain (Daly et al. 1987). These bees are mass provisioning, providing a ball of pollen and nectar to each egg prior to oviposition. Following foraging and oviposition a brood cell is capped with a wood pith septum and the process is repeated. Little is known about their biology but intranidal activities such as partition destruction and maternal grooming have not been reported in this tribe. Usually only one bee is found per nest entrance. Occasionally up to ten females are reported per nest, each occupying a separate branch and sharing a communal nest entrance (Daly et al. 1987). Nest observations and dissections have revealed solitary (single foundress) and communal (multiple foundress) nests, but none have demonstrated cooperative work on brood cells or reproductive castes (Flores-Prado et al. 2008).

The Xylocopini are the largest of the Xylocopinae, commonly referred to as the large carpenter bees. Xylocopini share the branched nesting architecture and mass provisioning described for Manueliini. Observations across numerous species have confirmed both solitary nests and multiple foundress colonies (Sakagami and Laroca 1971). X-ray photography and artificial nest manipulation have revealed a range from solitary (single female) to communal (casteless) to guarding (caste-like) intranidal behaviour (Gerling et al. 1981; Velthuis 1987). Solitary nests are either acquired or formed by a lone female. In some species mothers die in the first year and there is little interaction between generations (Michener 2000). Conversely, some species are polygynous with multiple related or

unrelated females occupying the same nest in separate branches year round. Multiple female nests are always branched with a lone female occupying each chamber and tending to it independently (Velthuis 1987). Polygynous colonies are communal with no cooperative work or reproductive castes. Xylocopine bees have attained a unique form of sociality aided by their remarkable longevity. In multiple female nests first year daughters remain in the nest as non-reproductive guards while their second year mothers monopolize foraging and oviposition. Xylocopini demonstrate caste-like division of labour, however it is distinctive in that non-reproductive 'workers' do not work but remain in the nest as guards (Hogendoorn and Velthuis 1999). 'Castes' in these bees represent ontogenetic stages rather than classes of individuals (Michener 1990b). Such delayed communal behaviour limits the social evolution of the Xylocopini to parasocial never attaining proper castes or cooperative eusocial behaviours.

The Allodapini are small carpenter bees that occupy dead broken stems and form their nest entrances via exposed pith. Nest construction involves forming a single burrow along the grain of the pith. Among the Hymenoptera, allodapines are unique in that brood are not enclosed in individual brood cells but reared in a communal chamber. Brood are progressively fed small amounts of pollen and nectar throughout development, rather than one mass-provisioned allotment typical to most other bees. This progressive rearing style requires continuous contact and care for immatures (Michener 1974). The allodapines are known to range from parasocial to eusocial species with no reversion to solitary life (Chenoweth et al. 2007). Most species are monogynous and produce daughters who stay at the natal nest to help guard and feed their siblings. Subsequently, one female occasionally monopolizes reproduction and nestmates remain as non-reproductive helpers establishing a eusocial colony. Conversely, other colonies have multiple reproductive females and contribute foraging effort as well as offspring to the communal burrow (quasisocial)

(Michener 2000). Allodapines are best known for their ongoing parental care, mutual tolerance and communal linear nesting chamber.

Finally, Ceratinini are a group of small slender carpenter bees largely resembling Manueliini, but with linear stem dwelling nesting habits similar to the Allodapini. Ceratinines are unique among the Xylcopinae in that not only do they require the exposed pith of a dead broken stem to form a nesting burrow much like the Allodapini, but also separate brood in cells using pith septa partitions. Ceratinini are mass provisioning bees providing all the nectar and pollen an immature will receive for development prior to oviposition. Following oviposition brood cells are capped with septa made of pith scrapings from the nest wall interior. This process is repeated in a serial manner. Ceratinini are the most socially polymorphic of the Xylocopinae. Most described species are solitary, but occasionally conspecifics form multiple female communal and even eusocial nests (Sakagami and Maeta 1995).

Social Evolution of the Ceratinini

A comprehensive phylogeny of the Ceratinini is not only desirable from a historical biogeographic point of view, but also provides a framework for examining the routes to sociality in the tribe. Once we understand the systematic routes and order of dispersal, a molecular phylogeny will provide a starting point for further exploration into the number of origins and potential losses of social behaviour in the Ceratinini. This molecular roadmap will help unify existing behavioural observations and as new taxa and behavioural data are revealed, they too can be incorporated. Given that the highest form of sociality has evolved in the sister subfamily Apinae, it is important to elucidate whether sociality is a basal trait of the Apidae including the Xylocopinae or a more a more recent and recurring event.

It has been suggested that subsociality (prolonged parental care) is a fundamental precursor or preadaptation to eusociality (Wilson 1971). The plesiomorphic subsociality found across Apidae and absent in the sister family Megachilidae will become fundamental to understanding the subsequent behavioural repertoires observed in each lineage. Some authors have suggested that understanding the genetic bases of social behaviours will fully and finally explain social evolution of the insect societies (Hunt and Amdam 2005). However, field observations and behavioural data suggest that although many species may possess behavioural precursors such as subsociality, it is the life history and ecological factors that promote and maintain sociality. An external phylogenetic context is imperative to distinguish the role of intrinsic genetic factors versus extrinsic environmental pressures on the evolution of sociality.

Part 2: Evolutionary Explanations of Altruism

Whether one measures biodiversity, biomass or behavioural complexity, eusocial insects are arguably the most abundant and specialized animals on the planet (Wilson 1971). Eusociality is characterized by overlapping generations, cooperative brood care, and reproductive division of labour (Batra 1966; Michener 1969). A typical eusocial colony is founded by a mated queen who provisions and lays a first brood of non-reproductive workers. First brood workers then forage to provision the queen's second, reproductive brood. These second brood reproductives mate and become the next year's queens. It is widely accepted that eusocial taxa arose from solitary antecedents (Wilson 1971; Lin and Michener 1972; Linksvayer and Wade 2005). In solitary species, offspring disperse and reproduce independently whereas eusocial workers remain at the natal nest and forgo reproduction to aid the queen in rearing siblings.

Levels of eusociality are categorized by reproductive skew (proportion of offspring produced by each female in the colony), which varies from no skew (reproduction is shared among nestmates) to complete skew (a single individual dominates reproduction) (Pamilo and Crozier 1996). In highly eusocial taxa, reproductive skew is complete; queens monopolize reproduction and workers are a sterile caste. Primitively eusocial taxa have incomplete skew as queens dominate reproduction but workers are a partially or potentially fertile caste (Michener 1974).

Evolutionists have long recognized the difficulty of explaining the existence of sterile castes by individual selection, in which an organism gains fitness by producing and raising its own offspring. Darwin himself stated that eusocial insect workers are "one special difficulty, which at first appeared to me insuperable, and actually fatal to my whole theory...from being sterile, they cannot propagate their kind" (Darwin 1859, p.236). To remedy this problem Darwin proposed that workers must have evolved through selection of the colony, but without the concept of Mendelian inheritance he failed to provide a mechanism for how this would work. Despite advances in genetics, the question persists: why would an individual sacrifice its own reproduction to help another reproduce? Moreover, the evolutionary steps required to go from solitary to eusocial life remain unclear (Anderssen 1984; Michener 1985; Michener 1990a; Wilson and Hölldobler 2005). Theory suggests that workers evolved as a result of intrinsic genetic relatedness and kin selection (Hamilton 1964), extrinsic manipulation and staying incentives (Crespi and Ragsdale 2000), or ecological constraints on independent nesting (Lin and Michener 1972). The relatedness theory focuses on helping behaviour evolving because closely related individuals stay together out of collective benefit ensuring the survival of kin that share genes identical by descent. The theory of maternal manipulation suggests that queens coerce the worker brood into remaining as subordinate helpers at the nest by limiting their body size during development and policing their

reproductive opportunities as adults through physical aggression. Finally, growing numbers of ecological studies have linked group living to constraints in species' biotic and abiotic environments and have found that resource limitation, climate and predation pressure can all play roles in selection for sociality.

Kin Selection and the Evolutionary Origin of Eusociality

Hamilton (1964) proposed that since colonies typically consist of related individuals, a sterile altruist could accrue inclusive fitness through helping related kin to propagate alleles identical by descent (IBD) to those in the altruist. Inclusive fitness is "the effect of one individual's actions on everybody's numbers of offspring ...weighted by the relatedness" (Grafen 1984). Based on this idea of inclusive fitness Hamilton further proposed that kin selection was the underlying explanation for the origin of eusocial behaviour. The idea that eusocial behaviour evolved as a result of increased inclusive fitness was formalized by Hamilton's Rule. According to Hamilton's Rule, individuals could sacrifice reproduction and still pass on more genes IBD when $\mathbf{r_k b > r_o c}$, where $\mathbf{r_k}$ is the relatedness of the altruist to the recipient, **b** is the number of related brood raised, $\mathbf{r_o}$ is the relatedness of an individual to its own offspring, and **c** is the number of offspring the altruist sacrifices by helping. If relatedness is high then the inclusive fitness benefits accrued by remaining at the natal nest to rear a relative's brood could outweigh the cost of forfeited reproduction.

Eusociality has arisen most frequently and with greatest complexity in the Hymenoptera, the ants, bees and wasps (Wilson 1971). Hymenoptera are haplodiploid, meaning that males are haploid and females are diploid. Daughters comprise half their mother's genetic makeup and all of their father's genes, whereas sons consist of half their mother's genetic makeup and have no paternal genes. Hence, daughters with the same father share three quarters of their genes on average. Since females are more related to their sisters

(r = 3/4) than to their own offspring (r = 1/2), female workers pass on more alleles IBD and incur greater inclusive fitness if they raise at least 2/3 as many sisters as replacements for their own offspring (Andersson 1984). Although workers sacrifice personal reproduction, they compensate by helping to raise close relatives which share their genes.

Recent findings indicate that haplodiploidy and 3/4 relatedness between sisters may have been of limited importance for the evolution of eusociality. For example, multiple mating (polyandry) is common in eusocial species (reviewed in Bourke and Franks 1995; Crozier and Pamillo 1996). Polyandry decreases average relatedness between sisters and dilutes the inclusive fitness benefits that support the forfeit of reproduction (Gadagkar 1991). Moreover, eusociality has been discovered in numerous diploid organisms including the termites (Wilson 1971), aphids (Stern and Foster 1997), gall forming thrips (Crespi 1992), naked mole rats (Jarvis 1981), ambrosia beetles (Kent and Simpson 1992), snapping shrimp (Duffy 1996), and flatworms (Hechinger et al. 2010). These discoveries suggest that factors other than haplodiploidy must play a larger role in explaining the origin of eusocial behaviour.

Maternal Manipulation as an Amendment to Kin Selection

Alternative models have suggested that sociality may have arisen through maternal manipulation (Reeve and Keller 1997; Crespi and Ragsdale 2000). Experiments have shown that primitively eusocial organisms are extremely aggressive toward non-nestmates and tolerant of nestmates (Gamboa et al. 1987; Wcislo 1997). Close observations of intranidal (within nest) queen-worker interactions have noted that queens are not only reproductively but also aggressively dominant to workers (Brothers and Michener 1971; Kukuk and May 1988; Pabalan 2000) and they have been observed physically coercing 'lazy' daughters into working (Breed and Gamboa 1977; Packer 1986b; Reeve 1992). With consistent behavioural

dominance between queens and workers, the evolution of eusociality could be more attributable to maternal coercion and less to do with kin selection.

In addition to reproductive division of labour, eusociality is also characterized by overlapping generations (Michener 1969). Thus, mothers and daughters (queens and workers) interact with one another. As sister-sister relatedness decreases, mother-daughter conflict emerges. Sisters sired by two fathers have reduced relatedness and incur higher fitness by producing their own offspring rather than raising their half siblings (Trivers and Hare 1976). However, queens still benefit from having non-reproductive workers to help raise their offspring. To combat decreased worker fidelity, mothers in some species coerce their daughters into forgoing reproduction. This includes producing daughters of reduced body size which are easily controlled and constant physical policing of their reproductive activity as adults. Through reduced offspring body size and physical brutality mothers are able to oppress workers and impede their daughters from attaining direct fitness opportunities as solitary foundresses (Crespi and Ragsdale 2000). In hymenopteran examples, mothers have complete control over the body size of their offspring by limiting provisions to a developing egg (Klostermeyer et al. 1973; Johnson 1988; Bosch and Vicens 2002). It has been suggested that smaller offspring are easier for the queen to physically control (Sakagami and Maeta 1977; Kukuk and May 1991; Packer and Knerer 1986; Dunn et al. 1998). However, small size does not significantly reduce helper efficiency (Lin and Michener 1972; Vogel and Kukuk 1994). Physical suppression of ovarian development in workers is frequently a result of aggressive behaviour (i.e. nudging and butting) by the queen (Brothers and Michener 1971; Michener 1990a; Pabalan et al. 2000). As long as size and physical manipulation do not impede the worker's ability to help produce sufficient numbers of related offspring, both the queen and the worker can benefit (Crespi and Ragsdale 2000). Maternal

manipulation is not a mutually exclusive alternative to kin selection because by definition, maternal manipulation involves close relatives.

Ecological Constraints and Selection for Group Living

In light of these recent discoveries, alternative explanations for the origin of eusocial behaviour have been proposed. Many authors have suggested that worker behaviour arose through ecological constraints such as predator and parasite pressure, in which females stay at the natal nest to guard siblings, decreasing brood mortality and total nest failure (Schwarz 1988; Cronin and Schwarz 1997; Martins 1999). Resource limitation has also been proposed as a mechanism limiting opportunities for independent nesting wherein females remain and work at the natal nest due to the scarcity of available nesting substrate or food resources elsewhere (Sakagami and Maeta 1977; Michener 1974; Michener 1985). In addition, comparative studies reveal that sociality typically traces geographic and climatic gradients within genera and species show an increasing level of sociality at lower latitudes and altitudes (Eickwort and Eickwort 1971; Packer 1990; Sakagami and Munakata 1972). Sociality is thought to be favoured by longer active breeding seasons and overlapping generations. However, exceptions reveal opposite patterns in some social arthropod lineages (Jeanne 1991; Schwarz et al. 1997; Furey 1998; Jones et al. 2007). Ecological factors vary across time and location, thus each population examined reveals an array of specific environmental constraints but no underlying conditions constant to all (reviewed in Purcell 2010).

Model Systems to Study Social Origins

In order to study the importance of kin selection, maternal manipulation and ecological constraints in the formation of altruistic behaviour, it is most informative if model organisms are socially polymorphic species exhibiting a range of solitary to social life. In

highly eusocial taxa (ants, termites, honey bees), workers are a sterile caste differentiated from reproductives during development; therefore these taxa offer no comparative material to determine the initial incentives that lead to their altruistic behaviour (Michener 1974). In primitively eusocial species (carpenter bees and sweat bees), females retain social plasticity into adulthood, capable of becoming a queen or a worker (Michener 1974; Michener 1990a). Thus, through detailed study of primitively eusocial taxa one can begin to resolve the relative importance of factors leading to helping behaviour.

The Small Carpenter Bees

The small carpenter bees (genus, *Ceratina*) are prime candidates to test ecological and genetic theories of social evolution. Small carpenter bees are found on all continents across a gradient of geographic and climatic environments and species exhibit the full spectrum from solitary to eusocial colony organization (Michener 1985; Michener 1990b). These species exhibit prolonged maternal care, nest loyalty and mutual tolerance among nestmates (Sakagami and Maeta 1977). Furthermore, some species are socially polymorphic with both solitary and social individuals in a single population (Michener 1985) providing the behavioural plasticity to test ecological constraints, kin selection and maternal manipulation hypotheses.

The Australian small carpenter bee, *Ceratina australensis* is of special interest to the study of social behaviour in bees. This species has previously been studied by Michener (1962). Michener noted that one nest contained two adult females and immature brood, but this single observation was inadequate to explain the nature or circumstance of the species' social potential. Detailed studies in North American ceratinines failed to observe a single multifemale, brood-rearing colony (Kislow 1976; Johnson 1988; Rehan and Richards 2010),

thus the opportunity to study behaviourally labile species contributes to our understanding of the social potential and behavioural plasticity among the small carpenter bees.

Research Aims

The research presented here aims to describe the social behaviour of previously uncharacterized small carpenter bees. This research investigates social potential in a range of *Ceratina* species, contrasting both intra- and interspecific social variation to test ecological and genetic theories for the formation and maintenance of social groups. More specifically, Chapter 2 presents the nesting biology and social structure of the Australian small carpenter bee, *Ceratina australensis*. Chapter 3 examines the role of ecological constraints and temporal variation in the reproductive success and social behaviour of the Australian small carpenter bees. Chapter 4 includes genetic relatedness to determine the role of kin selection and maternal manipulation in *C. australensis*. This chapter provides the first direct relatedness estimates for *Ceratina* colonies. Chapter 5 contrasts social behaviour and nesting biology of four small carpenter species from Borneo. Small carpenter bees are thought to be largely solitary, but this study shows sociality is recurrent across a variety of taxonomic groups. Chapter 6 combines all behaviourally characterized *Ceratina* species, as well as additional behaviourally unclassified species, representing taxa from every continent and ecological region to provide the first comprehensive molecular phylogeny of the small carpenter bees. Chapter 7 is a general discussion of the results obtained throughout the thesis and is also a review of the implications of this research for understanding social evolution.

Chapter 2:

Social polymorphism in the Australian small carpenter bee, *Ceratina (Neoceratina) australensis*

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INTRODUCTION

The origin of eusociality from solitary antecedents is one of the major transitions in evolution (Maynard Smith and Szathamáry1995). The highly eusocial termites, ants, wasps, and corbiculate bees all evolved sociality >65 million years ago and exemplify end-stages rather than origins of social behaviour (Thorne et al. 2000; Engel and Grimaldi 2005; Wenzel 1990; Michener 2007). Scrutinizing these highly social clades to infer the nature of the earliest insect societies is difficult, if not impossible, because transitional stages no longer exist, and solitary ancestors are hypothetical starting points with only derived highly eusocial end-points for study. Understanding transitions from solitary to social life requires a group of closely related taxa possessing both social and non-social species, ideally, with recent and repeated origins of sociality. The resulting contrasts would allow us to tease out the genetic, life history, and environmental factors that promoted or constrained the origins of sociality.

Compared to the advanced eusocial insects, more recent and labile social evolution is found in the halictine and allodapine bees (Schwarz et al. 2007), and these primitively eusocial taxa have provided insights into the evolutionary steps from solitary to social life. Extensive behavioural data and robust molecular phylogenies have revealed that evolutionary origins of eusociality are few, with three origins and 12 losses of eusociality in the halictines (Danforth 2002) and a single origin of sociality with no reversion to purely solitary life in allodapines (Chenoweth et al. 2007).

Early studies (Michener 1974; Wilson 1971) suggested that sociality had evolved within the bee tribe Allodapini and that its sister tribe, Ceratinini, was largely solitary. This suggested that extant allodapines may contain some interesting contrasts that could be used to infer early stages in the evolution of true sociality, whereas ceratinines represented an origin of extended mother-brood contact, with sporadic tolerance of adult daughters by still-

reproductive mothers. However, it is now known that sociality is an ancestral trait for Allodapini, with no known losses of sociality (Chenoweth et al. 2007), whereas an increasing number of studies indicate that multi-female nesting during brood rearing may be widespread among Ceratinini (Sakagami and Maeta 1977, 1987, 1995; Rehan et al. 2009).

All ceratinines studied to date are subsocial (sensu Michener 1969; Wilson 1971; Tallamy and Wood 1986), with prolonged maternal care and mother-offspring interactions (Sakagami and Maeta 1977; Michener 1990; Rehan et al. 2009; Rehan and Richards 2010). In addition, some species are socially polymorphic, with both solitary and social nests in the same population (Sakagami and Maeta 1987; Michener 1985). Solitary nests are attended by a single adult female while social colonies usually contain two, but occasionally three to four, adult females (Michener 1990; Rehan et al. 2009). Reproductive division of labour and social polymorphism is recurrent among the Old World subgenera, *Neoceratina* (Rehan et al. 2009), *Ceratinidia* (reviewed in Michener 1985; Rehan et al. 2009), *Pithitis* (Rehan et al. 2009), and possibly *Ctenoceratina* (Daly 1988). However, most subgenera and the vast majority of species have not been studied. The ceratinines may therefore provide comparative material that can help elucidate the origins of multi-female nesting as well as reproductive differentiation among nestmates, in the way that allodapines were once thought to provide.

Ceratina are well represented on all continents except Australia where there is a single described species, *C. (Neoceratina) australensis*. The life history and social potential of *C. australensis* was previously described, based on monthly sampling from July 1958 through February 1959 comprising a total of 38 nests, with a single nest in which brood were being reared by two females (Michener 1962). In the absence of larger sample sizes the life cycle, developmental rate of immatures and the significance of two-female associations

remains speculative. Here we use nest collections of *C. australensis* over a period of 20 months, covering winter, spring, and summer periods to investigate colony phenology, social nesting, reproductive hierarchies and brood productivity. We use these data to discuss factors that influence colony formation and behavioural preadaptations in incipiently social taxa.

METHODS

A total of 612 *C. australensis* nests were collected from dead broken stems of giant fennel (*Ferula communis)* in and around the shire of Warwick in the warm temperate zone of southern Queensland, Australia (28° 13' S 152° 02' E, 480m elevation). Fennel stalks were found along rural roadsides adjacent to grain and cattle farms in Warwick and surrounding areas. Nests were collected prior to 0700 h to ensure that bees had not commenced flight activity for the day, so that all nest occupants would be present. Stems were broken at the base and the nest entrances sealed with masking tape for transport on ice to the lab, where they were stored at 5°C until examined. Nests were split lengthwise and contents recorded, including number of brood cells, number of live brood, developmental stages of brood, number and location of adult bees, and overall nest appearance. Nest lengths were measured using digital calipers (accuracy ± 0.01 mm). Collections were undertaken at four times of year: winter (July 2007 and 2008), early spring (October 2007 and 2008), late spring (December 2007 and 2008), and late summer (February 2008 and 2009).

Nests were categorized according to the developmental stages of Daly (1966) and Rehan et al. (2009). *Hibernacula* contain faecal pellets or pollen residue with darkened interior walls from the previous breeding season and may contain one to six adult bees. *Founding* nests contain eggs, larval provisions or brood cells and are formed in newly

excavated pith. *Active brood* nests contain pollen masses with eggs or small larvae. In *full brood* nests, the cell closest to the nest entrance contains a larva or pupa. Only full brood nests were used to evaluate the number of live brood and clutch size (the number of brood cells in the nest). *Mature brood* nests contain callow offspring and adult bees, but no pollen provisions or immature offspring. In addition to these stages, nests were categorized as new versus reused. *New nests* have clean walls devoid of pollen stains and faecal pellets while *reused nests* have darkened walls with pollen and/or faecal stains from previous provisioning and brood rearing in that twig.

Brood were removed from the nest and reared in the lab at a temperature ranging between 23-25°C in 200µl microcentrifuge tubes with an air hole inserted in the lid. Each immature was observed daily to determine the number of days spent in each of the 18 developmental stages previously identified for ceratinine bees (Daly 1966; Rehan et al. 2009).

Adult females were assessed in terms of body size and reproductive status. Head width was measured across the widest part of the head to the outer margins of both compound eyes. Wing lengths were measured along the costal vein from the base of the wing to the proximal tip of the stigma. Wing length and head width were linearly correlated ($r = 0.812$, $n = 129$, $p < 0.0001$). In addition females were weighed using a Mettler analytical balance (accuracy 0.001 mg). Live weight and head width were linearly correlated ($r = 0.787$, $n = 94$, $p < 0.0001$), therefore head width was used as a proxy for body size for adult females. Wing wear was scored to assess foraging effort (Cartar 1992). Bees with no nicks or tears on the apical margins of both forewings received a wing wear score of zero, and bees with the apical margin of both forewings completely worn to shreds received a wing wear score of five. Adult females were dissected to determine reproductive status. Ovary size was measured as

the sum of the lengths of the three largest terminal oocytes (accuracy ± 0.01 mm). Insemination status was determined by the presence or absence of sperm in the spermatheca.

In this study solitary nests contain a single foundress and social colonies contain two foundresses. Social nests were conservatively identified when two adult females were found within nests with reproductive activity (active and full brood nests). However, hibernacula, founding nests and mature brood nests were not recorded as social colonies as these represent pre and post-reproductive assemblages which could potentially disperse prior to reproduction.

Statistical analyses

Descriptive statistics, goodness-of-fit tests, t-tests, ANOVA, and resampling statistics were carried out using SAS version 9.1. Data were assessed for normality and when response variables were not normally distributed; continuous measures were replaced with ranks for non-parametric statistics. Measures were combined across samples for all statistical analyses.

RESULTS

Frequency of social nesting

Of 612 nests collected over two years, 262 were reproductive (active and full brood) nests, and 36 (14%) of these contained two adult females with the remainder containing a single adult female. Solitary and social nests were found in neighbouring fennel stalks and were indistinguishable except for the number of adult females inside. Social colonies were collected in early spring, late spring and summer. Collections of social nests showed that they were at stages similar to those of solitary nests collected at the same time (Table 1).

Colony cycle

Ceratina australensis immatures develop from egg to adulthood in about 34 days (Table 2), and the maximum age difference between youngest and oldest offspring within a given nest was 21 days. Therefore the maximum time required to complete a brood should be about 55 days. Based on nest collections (Table 1) and brood developmental rates (Table 2), the seasonal phenology of the species is depicted in Figure 1 and described below.

In winter (July collections), all nests found were hibernacula, about one-third being newly constructed and two-thirds being reused nests. Hibernacula contained on average two adult females per nest (range 1-6 females). No males or immatures were found in hibernacula.

In early spring (October collections), all nests collected were founding nests and active brood nests, so provisioning and oviposition of brood were at an early stage. The majority of spring nests were newly constructed, with about 25% (41/164) being reused. By early summer (December collections), most (92/118, 78%) nests were in the active and full brood stages, but there were also a few founding (21/118, 18%) and mature brood (5/118, 4%) nests as well. As in early spring, the majority of early summer nests were of new construction.

The few founding nests collected in early summer likely represent early production of a second brood. This second brood was produced mainly in mid-summer (February collections) as shown by the abundance of founding and active brood nests collected (Table 1). These cannot have been first brood nests because, as noted above, it takes less than two months to complete a brood. The proportions of new and reused nests were similar in

summer and late spring collections, i.e. the proportion of newly constructed nests was similar for first and second brood (Fisher's exact test, $p = 0.46$).

Overwintering females (July collections) were a mix of unworn (72%) and worn (28%) individuals (Table 1). Worn females in hibernacula must have been foragers during the previous summer and must therefore have been first brood females produced in spring. Females that were unworn most likely were second brood females produced over the previous summer. Therefore, hibernacula contained both first and second brood females.

The considerations above suggest that there are two brood production periods, Brood 1 and Brood 2. Early spring collections revealed that 12% of nesting females were heavily worn (wing wear score >3), even though their nests were only in the founding and active brood stages. This implies that worn females were nesting for the second time whereas unworn females were nesting for the first time. Likewise, 25% of nesting females from summer collections were heavily worn, so again, these must have been re-nesting while unworn females were nesting for the first time. In other words, individual females followed one of the following nest phenologies: females could produce their first brood in spring and then a second one in summer, or, if they emerged in late spring, produced a first brood in summer and then a second one in spring after overwintering. Since the proportions of unworn and worn females did not vary between new and reused nests ($\chi^2_1 = 2.25$, $p = 0.32$), there was no correlation between female age and nest reuse patterns.

Maternal care and longevity

Mothers inspect brood during their development. At the time of nest opening females were found inside brood cells amongst loose pith partitions and inspecting immature bees in

7/245 (3%) of attended active and full brood nests. Mothers were found inspecting innermost and outermost brood cell positions. However, 238/245 (97%) mothers were found guarding the nest facing backwards with their abdomen blocking the entrance in active and full brood nests and all cell septa were found intact in nests when the mother was discovered at the nest entrance. This suggests that mothers inspect brood cells on occasion but must reconstruct brood cell partitions following inspection.

Females are long lived and nest loyal as evinced by adult females found in 99% (155/157) of active brood, 86% (90/105) of full brood, 47% (36/76) of mature brood, and 87% (530/612) of all nests. The mean period from commencement of brood rearing to maturation of the brood is 34 days (Table 1). Therefore, adult females were likely to have lived for at least one year prior to collection with their complete brood, considering the duration of overwintering and brood production.

Reproductive hierarchies in social colonies

In the absence of observation nests we examined reproductive differentiation and its possible determinants using colony census and dissection data from females collected in active and full brood nests. Solitary females were used as a point of comparison to determine the possible roles of females in social colonies.

First, reproductive differentiation between nestmates in social nests was addressed by examining the distribution of reproductive development in social and solitary nests (Fig. 2). Given the range of ovary sizes across the population as a whole, we asked if reproductive differentiation between social females was greater than would be expected among randomly drawn pairs of solitary females. To do this we used a Monte Carlo resampling technique

(Sokal and Rohlf 1995). The mean absolute difference in ovarian sizes between 25 pairs of females in social colonies was calculated. We then randomly selected 25 pairs of females without replacement from the solitary nests and calculated their mean differences in ovary size. This procedure was repeated 1000 times to produce a null distribution of differences among randomly selected solitary females to which we compared the observed mean difference between females in social colonies. Only four of the 1000 simulated mean ovary size differences were greater than that observed in the social colonies, indicating that the difference in ovary size is greater in social colonies than would be expected by chance. Two-sample t-tests comparing ovary sizes of solitary females with first ovary size-ranked social females revealed no difference ($t_{79,25} = -0.934$, $p = 0.17$), while solitary and second ovary size-ranked social females were significantly different ($t_{79,25} = 3.44$, $p = 0.02$).

Second, we addressed whether ovary size scales with body size independently of social interactions. To do this we compared head width to ovary size in solitary females from reproductive (active and full brood) nests. There was no relationship between body size and ovary size ($r^2 = 0.03$, $n = 79$, $p = 0.102$). Given the lack of body-size scaling of ovary size in solitary females we compared body size and ovary size in social nests. For social colonies we ranked individuals according to ovary size and compared absolute body size between first (1.48 ± 0.07 mm) and second (1.46 ± 0.07 mm) ovary size-ranked social females in the population. This showed no significant difference (paired t-test, $t_{25} = 1.43$, $p = 0.16$). We then ranked social individuals according to body size and ovary size and tested these two ranks for independence for all samples combined and found no dependence between ranks (Fisher's exact test, $p=0.24$).

Thirdly, we examined ovary size as a function of wing wear. We tested whether wing wear differed between first and second ovary size-ranked females in social colonies. There

was a significant difference (Fig. 3; paired t-test, $t_{25} = 5.36$, $p < 0.0001$) in wing wear between primary (3.31 ± 0.93 mm) and secondary (0.48 ± 0.35 mm) ovarian size-ranked social females. Two sample t-tests again revealed that secondary ovary size-ranked social females had significantly less wing wear than solitary (2.47 ± 1.68 mm) females ($t_{25, 79} = -6.41$, $p < 0.0001$), but solitary and primary ovarian size-ranked social females did not differ from each other ($t_{79, 25} = -0.01$, $p = 0.50$).

The significant relationship between ovary size rank and wing wear prompted additional exploration of wing wear as a predictor of reproductive differentiation. First, wing wear variation between nestmates was addressed by categorizing females as having either the greater or lesser wing wear compared to their nestmate. Much like the ovary size analyses above, we examined wing wear as a function of ovary size. We tested whether ovary size differed between primary and secondary wing wear score-ranked females in social colonies. There was a significant difference in ovary size between primary (1.94 ± 0.58 mm) and secondary (1.00 ± 0.40 mm) wing wear-ranked social females (paired t-test, $t_{25} = 6.93$, $p < 0.001$). Two sample t-tests revealed that secondary wing wear-ranked social females had significantly smaller ovaries than solitary (1.56 ± 0.62 mm) females ($t_{25, 79} = -3.30$, $p = 0.001$), but solitary and primary wing wear-ranked social females did not differ from each other ($t_{25, 79} = 1.7$, $p = 0.13$).

We then addressed whether wing wear scales with body size independently of social interactions. To do this we compared head width to wing wear in solitary females from reproductive (active and full brood) nests. There was no correlation between body size and wing wear ($r = 0.08$, $n = 79$, $p = 0.48$). Given the lack of body-size scaling of wing wear in solitary females we compared relative wing wear and body size in social nests. For social colonies we ranked individuals according to wing wear and compared absolute body size

among solitary (1.47 ± 0.07 mm), primary (1.48 ± 0.07 mm) and secondary (1.45 ± 0.07 mm) wing wear-ranked social females in the population. There was no significant difference in absolute body size between primary and secondary wing wear-ranked social females (paired t-test, $t_{25} = 1.65$, $p = 0.11$). Two sample t-tests further confirmed there was no difference in body size between solitary and primary wing wear-ranked social females ($t_{79, 25} = 0.10$, $p = 0.92$) or solitary and secondary wing wear-ranked social females ($t_{79, 25} = 1.64$, $p = 0.11$).

Overall, colony census and dissection data from adult females collected in reproductive nests indicate that: (i) there is bimodality in ovary size and wing wear among social females, (ii) body size is a poor indicator of both wing wear and ovary size in social colonies, (iii) females with larger ovaries tend to have greater wing wear, and (iv) solitary females are similar to social first ovary size-ranked female in both ovary size and wing wear patterns.

Nest architecture and brood productivity

Of the 612 nests collected, 204 or 33% were reused and 408 or 67% were newly founded nests. Nests lengths ranged from 9 to 245 mm. New nests were 80.3 ± 31.9 mm and reused nests 83.2 ± 29.6 mm in length and there was no significant difference between these means ($t_{201,121} = 0.811$, $p = 0.42$). Reused nests were soiled throughout, suggesting that nests were not lengthened prior to reuse.

To determine the effect of nest reuse on reproductive success we compared the number of brood cells provisioned in new and reused full brood nests. There was no significant difference in clutch size between new (5.61 ± 2.96) and reused (5.42 ± 3.0) nests ($t_{61,30} = 1.11$, $p = 0.27$). In addition, there was no significant difference in the number of live

brood ($t_{61,30} = 0.41$, $p = 0.68$) between new and reused complete nests. This suggests that females which rear brood in reused nests are no more fecund than those rearing brood in new nests.

Social colonies were found predominantly in reused nests (35/36 colonies) suggesting that cohabiting females remain in previously used nests rather than co-found new nests. There was no significant difference in clutch size between solitary and social full brood nests (Fig. 4; $t_{99,6} = 2.45$, $p = 0.87$). Complete brood mortality was not observed in social colonies (0/6), but was found in 7/99 (7%) of solitary full brood nests, but these proportions were not significantly different (Fisher's exact test, $p = 0.5114$). However, the number of social full-brood nests here is small ($N = 6$) and it seems likely that some of our single-female full brood nests had initially started as social nests but susequently one nestmate had died prior to sampling. Such colonies are not detectable in our analyses, but it seems very unlikely that females suffer zero mortality between the start and finish of brood rearing. When analyses are based on all nests with brood (i.e. active and full brood nests), there was a significant difference in the proportion of live brood between solitary and social reproductive colonies (Fig 4; $\chi^2 = 6.74$, $p = 0.0094$). For these nests mean per-nest brood mortality was 14% for solitary females and 2% for social nests.

DISCUSSION

Our study found a low level of social nesting in *Ceratina australensis*, with only about 14% of the 262 colonies with active or full brood containing more than one adult female. This contrasts with some Asian species where rates of multi-female nesting were as high as 25% (Sakagami and Maeta 1987; Rehan et al. 2009), and also differs from some

holarctic studies where females have never been found to nest socially during brood rearing (Malyshev 1913; Kislow 1976; Rehan and Richards 2010). However, we note that our estimate of 14% is likely to be an underestimate, given that any colonies in our samples that began as social nests but where one female died prior to sampling would have been counted as a solitary nest.

In the following discussion we compare our results to other studies to consider life-history traits in ceratinines that may facilitate or constrain multifemale nesting during brood rearing. We then discuss reproductive differentiation and the nature of social colonies in *Ceratina*, and finish by asking whether low levels of sociality could represent a transitional stage to more frequent colony formation in the ceratinines.

Maternal behaviour and social preadaptations

The transition from solitary to eusocial life requires behavioural precursors from which overlapping generations, cooperative brood care and reproductive division of labour evolve. Such preadaptations include prolonged maternal care, maternal longevity and mutual tolerance (Wilson 1971; Lin and Michener 1972; Michener 1985). Mothers of all studied *Ceratina* species demonstrate prolonged parental care and guard their brood throughout development (Kislow 1976; Sakagami and Maeta 1977; Rehan et al. 2009). All studied ceratinines also exhibit high frequencies of maternal survival and cohabitation with mature brood (Rau 1928; Sakagami and Maeta 1977; Johnson 1988; Rehan et a. 2009; Rehan and Richards 2010). In this study, *C. australensis* adult females were found in 94% of nests with immature brood indicating nest loyalty and longevity in this species as well, both requisite for social cohabitation.

Maternal longevity is thought to influence brood survival as mothers protect their brood by acting as guards at the nest entrance (Kislow 1976; Sakagami and Maeta 1977). In this study we occasionally observed females inspecting brood cells. This behaviour is recurrent in ceratinines (Kislow 1976; Sakagami and Maeta 1977; Rehan et al. 2009, Rehan and Richards 2010). Further interaction with brood is indicated by the relatively high frequency (47%) of mother-offspring cohabitation in mature brood nests. This subsocial interaction provides an early opportunity for contact and communication between mothers and offspring. This is in contrast to solitary bees that provision and seal brood cells and have no further contact with their developing offspring.

Female dispersal and social nesting

Dispersal prior to brood rearing has a very strong potential to limit social nesting since it breaks up kin groups. In the allodapines, cofounding of new nests by relatives has evolved only once, in the genus *Exoneura* (Schwarz et al. 2007). In all other species new nests are solitary founded and in most of these species the modal colony size is one (Schwarz et al. 2007). In our study only one of the 36 social *Ceratina australensis* colonies was in a new nest, suggesting that female dispersal is likely to constrain social nesting. Cofounding in natural populations of other *Ceratina* species is also very rare. *Ceratina australensis* overwinters in both newly founded and reused stems and the only other ceratinine reported to also disperse and found new nests in autumn is *C. (Ceratinidia) flavipes* (Kidokoro et al. 2003, 2006). In both these species, autumnal dispersal should therefore lower the potential for social nesting in spring, and for *C. flavipes* only rarely (0.1% of nests collected) forms social colonies in the wild (Sakagami and Maeta 1987). Conversely, *C. japonica* (a sympatric sister species of *C. flavipes*) does not disperse prior to overwintering and

frequently forms social colonies in reused nests (63/203 or 31%) but rarely in newly founded nests (3/230 or 1.3%) during the spring brood rearing season (Sakagami and Maeta 1987). Nest reuse is associated with social nesting of *C. (Ceratinidia) okinawana* as 57/276 or 14% of reused nests and only 1/133 or <1% of newly founded nests contained a multi-female association (Sakagami and Maeta 1989). Likewise, in *C. (Ceratina) megastigmata* 4/5 multi-female colonies were found in reused nests (Katayama and Maeta 1979). *Ceratina (Zadontomerus) calcarata* is another well studied ceratinine that has never been observed forming social colonies and does not reuse nesting substrate (Kislow 1976; Johnson 1988; Rehan and Richards 2010). These data suggest social nests predominantly arise when females stay in a natal nest rather than joining or initiating a new nest.

Reproductive differentiation in social colonies

Behavioural differentiation among nestmates is pivotal to eusociality and a division of labour has been found in several bees thought to be incipiently social (Sakagami and Maeta 1987; Wcislo 1997; Jeanson et al. 2005). Social colonies of *C. australensis* contain only two females, and our data indicate that one female takes on both foraging and reproductive behaviour, while the second female has reduced ovarian development and wing wear suggesting neither reproduction or foraging activity. This suggests that the reproductive female will only tolerate the presence of a nestmate if that nestmate is non-reproductive, but the non-reproductive female does not seem to take on any foraging duties. We therefore need to ask why a non-reproductive female is tolerated, and why that female should forgo reproduction to remain as a non-reproductive, non-foraging nestmate. The social primary may tolerate the secondary female at the natal nest even though she does not contribute foraging effort as the mere presence of the secondary might contribute to the colony by

guarding brood while the primary reproductive is away from the nest. In addition, the social secondary may be a hopeful reproductive waiting to inherit the nest site from the social primary. This situation arises in social nests of some *Xylocopa* species (Hogendoorn and Velthuis 1993, 1995; Steen 2000) in which the dominant female is both the primary forager and the primary reproductive while the secondary female remains at the nest acting as a guard waiting for nest inheritance and supersedure. Other examples of auxiliary females remaining at the nest are found in some allodapine species where females remain at the nest in wait of future reproduction (reviewed in Tierney and Schwarz 2009).

Body size is often a strong predictor of dominance in bee species without morphological castes (Batra 1966; Michener 1974; Packer 1986; Hogendoorn and Velthuis 1999). The association between reproductive differentiation and size difference is well documented in social nests of three Japanese species *Ceratina (Ceratinidia) japonica*, *C. (Ceratinidia) flavipes*, and *C. (Ceratinidia) okinawana* (Sakagami and Maeta 1984, 1987, 1989). Greater head width differences between females were associated with greater reproductive skew in these three species. In eusocial and semisocial colonies of these species the larger female took on guarding and primary reproduction while the smaller female took on a foraging non-reproductive role. When size difference was slight reproductive skew was incomplete and quasisocial nests, in which both females are reproductive, were most common. In *C. australensis* size based reproductive dominance was not apparent. Size variation between females did not predict reproductive status as equal proportions of first and second body size-ranked females were reproductive.

Age is an additional predictor of reproductive differentiation among nestmates (Hogendoorn and Velthuis 1999). Eusocial colonies require overlapping generations, usually in which the mother is dominant to her daughters. Conversely, reproductive dominance in

semisocial associations may be attributable to a few days, if not hours, difference in eclosion among sisters (Schwarz and O'Keefe 1991). In the absence of prolonged nest observations it was difficult to assess the age of bees from nest collections in our study as age estimates from wing wear scores are confounded with foraging effort. Social primaries were worn and secondaries were not. Therefore, whether nests contain semisocial sisters or eusocial mother-daughter associates remains unknown. Future study including observation nests and/or genetic data should elucidate the age differentiation and status of each female in social colonies.

Brood productivity and social benefits

Two benefits of cooperative nesting have been identified for allodapine bees: (i) increases in per capita brood production, and (ii) prevention of total brood failure (Schwarz et al. 2007). In our study social colonies were no more fecund than single foundress nests suggesting that the additional female did not contribute to brood rearing. In general, social secondaries had weakly developed ovaries and were not active foragers as their wings were unworn. Despite the absence of foraging behaviour by social secondaries their presence could possibly contribute toward nest defence, either actively by blocking the nest entrance or passively by mere presence. We found no statistically significant increase in total brood size of social compared to solitary nests, so that per capita brood benefits are clearly not present in *C. australensis*. Although we found higher rates of total brood loss in solitary nests, this difference from social nests was not statistically significant. Overall brood mortality was limited in this species. Our results therefore raise two important questions regarding sociality in *Ceratina*: (i) why do we not see the benefits of social nesting that are evident in most allodapines; and (ii) given the lack of apparent benefits in *C. australensis*, why do we see the

low level of social nesting at all, given that secondaries are seemingly non-reproductive? Understanding these two issues is critical for discerning why the preconditions for sociality can evolve, but not then facilitate the evolution of eusociality.

Given the lack of apparent benefits to group living it is a wonder why social colonies remain in this species. The sister tribe Allodapini provides many examples of life history and ecological traits that seem to select for group living. The combination of progressive provisioning and the omission of brood cell septa leave immatures vulnerable to starvation in the absence of continuous care as well as exposure to predation and parasitism (Schwarz et al. 2007; Zammit et al. 2008). Sociality in the allodapines therefore seems to provide a selective advantage over solitary life, concordant with their ubiquitous sociality with no reversions to purely solitary life (Chenoweth et al. 2007). Conversely, sociality in the ceratinines may not be so advantageous given their mass provisioning and construction of brood cells (Michener 1974) requiring shorter durations of parental care and providing at least partial protection from predators and parasites.

It is thought that nest sharing evolved in bees and wasps because of the benefit of having more than one female available to defend the nest (Lin and Michener 1972; Michener 1974). Most species of sphecid wasps are solitary but one species, *Cerceris antipodes,* forms multiple female colonies which experience lower parasitism rates than solitary conspecifics (McCorquodale 1989). Likewise, in the sweat bee, *Megalopta genalis,* multi-female nests experience less brood parasitism (Smith et al. 2003) and higher brood survival rates (Smith et al. 2007) than solitary nests from the same population. We found a slight decrease in brood loss between solitary and social nests and the observation of total brood loss in solitary colonies suggests a selective advantage for social colonies during periods of extreme parasitism pressure.

Taken together, nearly all social nests result from nest-reuse and it seems likely that these nestmates are related. The near absence of newly founded social nests, in *C. australensis* and other socially polymorphic ceratinines suggests that sharing a nest results from remaining at the nest rather than finding or founding a new nest. That suggests that kinship is important for sociality, and that means that indirect fitness benefits are important for sharing a nest. In addition, we found evidence that sharing a nest lowers rates of brood mortality, so that may be one source of indirect fitness, but there was no increase in per capita brood production. However, we also found that rates of brood loss in solitary nests were about 14%, but close to zero% for social nests. Because clutch sizes are the same for social and solitary nests, the benefits for the social secondary can at most be 0.14. Such a small value should strongly curtail altruism. This might help explain the rarity of social nesting in this species, but it still requires that costs for a social secondary must also be very small. This could be the case if social secondaries merely delay the onset of their brood rearing, and this does not lower the potential number of brood they can rear. The remarkable longevity of ceratinines supports the feasibility of delaying reproduction for a few months with negligible costs for social secondaries.

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Table 1: Sample size and developmental stage of *Ceratina australensis* nests collected in Warwick, Queensland. Nest category notations are as follows: H = hibernacula, FN = founding nest, AB = active brood nest, FB = full brood nest, MB = mature brood nest, - = not applicable.

		Solitary Nests				Social Nests			
Collection	Nest	Nest appearance		Status of foundresses		Nest appearance		Status of foundresses	
period	category	New	Reused	Unworn	Worn	New	Reused	Unworn	Worn
July (winter)	H	25	42	82	32	-	-	-	-
October (early spring)	FN	88	23	99	12	-	-	-	-
	AB	35	11	37	8	0	7	7	7
	FB	0	0	-	-	-	-	-	-
	MB	0	0	-	-	-	-	-	-
December (early summer)	FN	10	11	16	5	-	-	-	-
	AB	26	7	23	10	0	6	8	4
	FB	47	2	31	11	0	4	4	4
	MB	1	4	1	3	-	-	-	-
February (summer)	FN	70	5	65	10	-	-	-	-
	AB	40	8	40	7	1	16	24	10
	FB	33	17	26	16	0	2	1	3
	MB	32	39	25	7	-	-	-	-

Table 2: Developmental rates of immature brood of *Ceratina australensis*. Eggs take on average three days to hatch and begin feeding on pollen mass (pb= pollen mass). Larval stages describe larva length compared to pollen mass (1/3-Full grown larva). Prepupae have consumed their entire pollen mass and defecate becoming more slender than younger larvae. Pupal stages (White-Black) describe eye pigmentation changes. Pupal stages (1/4 – Fully pigmented) describe body pigmentation observations ¼ pigmented through fully pigmented. Once fully pigmented the bee sheds one final molt becoming an adult.

	Stage	Mean (days)	SD (days)	n
Egg	egg	3.00	1.41	4
Larva	1/3-2/3 pb	2.10	1.00	10
	2/3-7/8 pb	1.10	0.23	10
	1 X pb	1.69	0.50	13
	1.5 X pb	1.44	0.50	18
	2 X pb	1.92	1.63	24
	Small bit pb	2.15	0.95	27
	Fully grown larva	2.11	1.50	36
	Prepupae	4.29	2.38	61
Pupa	White	1.39	0.58	84
	Pink	1.52	0.58	89
	Red	1.61	1.29	83
	Brown	2.06	0.96	94
	Black	2.01	0.63	98
	1/4	1.22	0.25	85
	1/2	0.99	0.25	92
	3/4	1.25	0.96	90
	Fully pigmented	1.84	1.54	93
	Total	33.73	1.69	1011

FIGURE CAPTIONS

Figure 1: Bivoltine colony cycle of *Ceratina australensis* in southern Queensland, Australia. Females overwinter (May to August) in hibernacula. In early spring (September-October) females disperse and found nests or reuse hibernacula. Mid-spring (October-November) females forage and provision brood cells. Late spring (November-December) provisioned brood mature in the nest and eclose as callow adults. Offspring emerge and mate at this time. Following emergence of the spring brood a second brood is initiated in early summer (January). Nest construction or reuse and brood cell provisioning span the summer months (January-February). Come autumn (March-April) the second brood offspring eclose. Callow offspring remain at the natal nest or emerge and re-nest in newly founded twigs for overwintering.

Figure 2: Comparison of reproductive status among *Ceratina australensis* females from active and full brood nests.

Figure 3: Box-plots of wing wear scores to ovary size ranks. Solitary females, N = 79; social primaries, N = 25; social secondaries, N = 25. Circles represent outliers.

Figure 4: Mean brood production and proportion of live brood in social and solitary full brood nests of *Ceratina australensis*. Solitary nests, N = 99; social nests, N = 6. Social nests produce equal numbers of offspring as solitary nests. Solitary nests have fewer brood surviving to adulthood than social nests.

Figure 1:

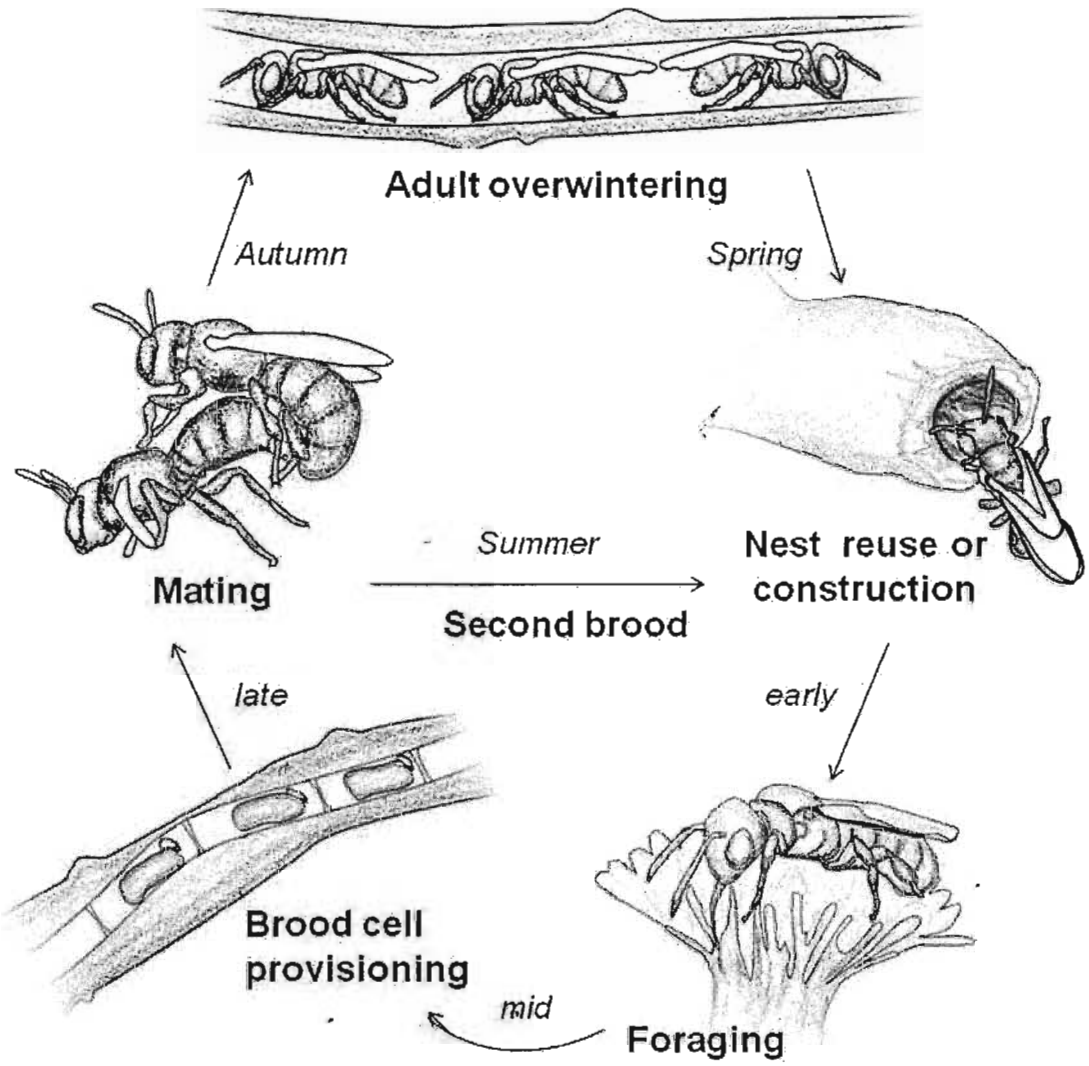

Adult overwintering
Autumn
Spring
Mating
Summer
Second brood
Nest reuse or construction
late
early
Brood cell provisioning
mid
Foraging

Figure 2:

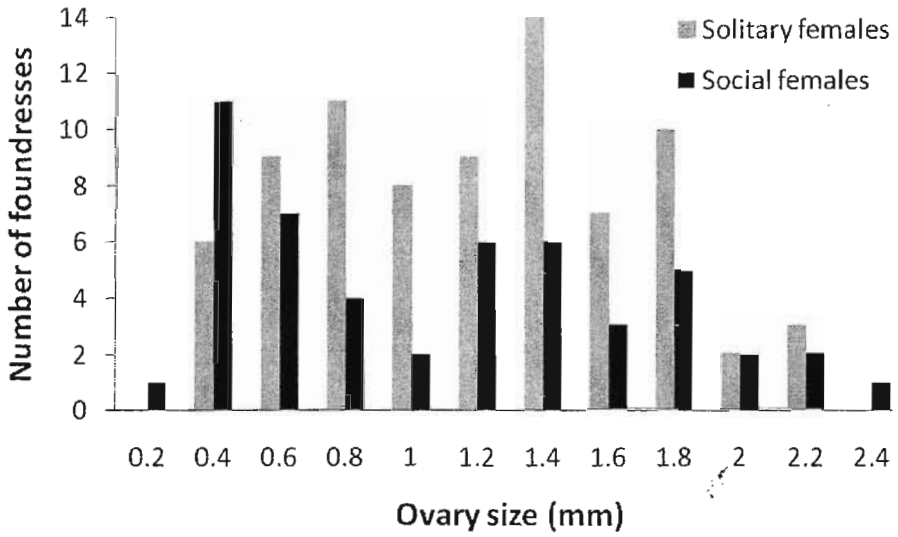

Solitary females
Social females
Number of foundresses
0
2
4
6
8
10
12
14
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
2.4
Ovary size (mm)

Figure 3:

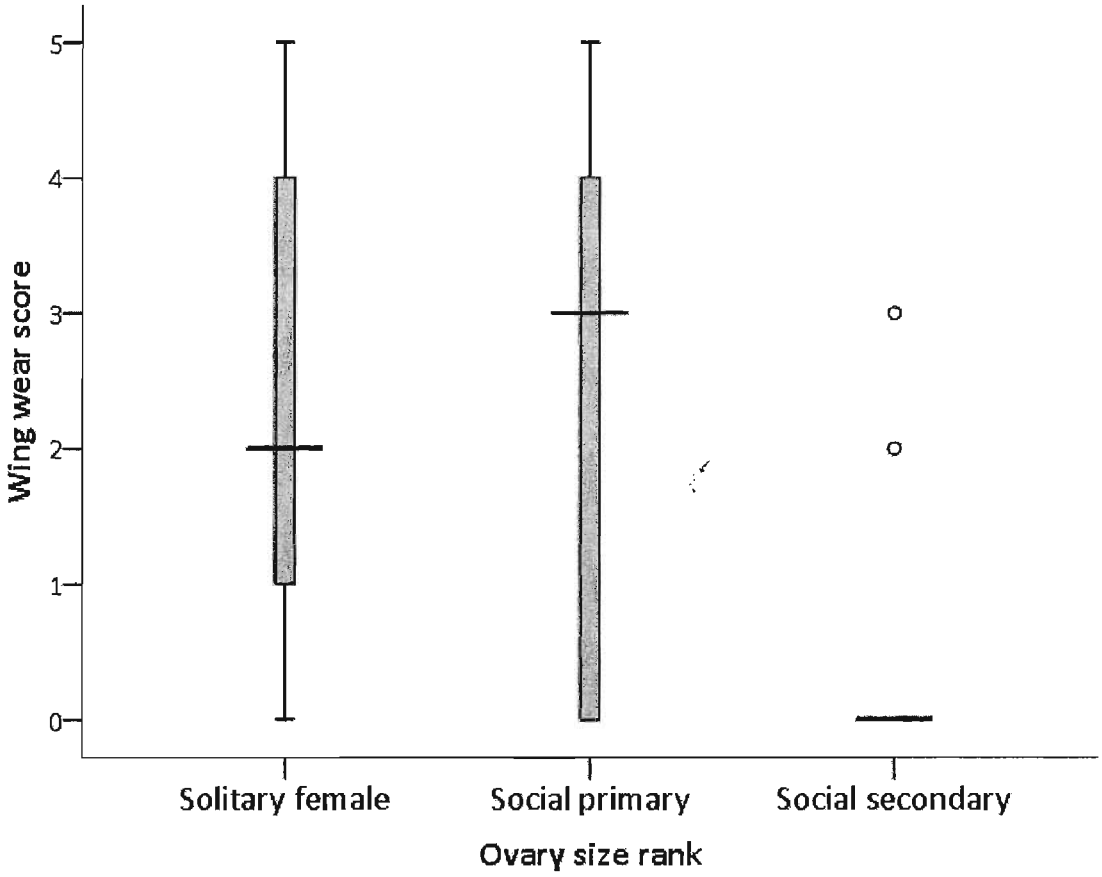
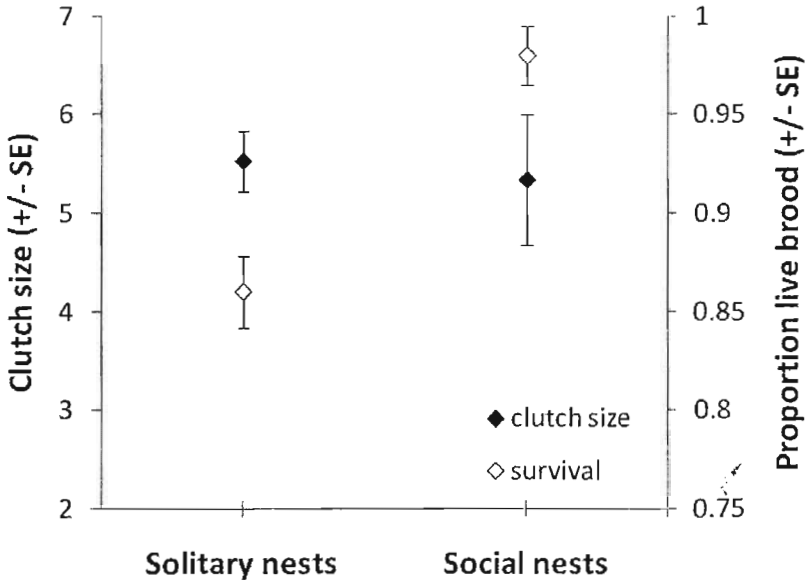

Wing wear score
5
4
3
2
1
0
Solitary female
Social primary
Social secondary
Ovary size rank

Figure 4:


7
6
5
4
3
2
Clutch size (+/- SE)
1
0.95
0.9
0.85
0.8
0.75
Proportion live brood (+/- SE)
clutch size
survival
Solitary nests
Social nests

Chapter 3:

Fitness consequences of ecological constraints and implications for the evolution of sociality in an incipiently social bee

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INTRODUCTION

Environmental conditions have the potential to greatly influence the survival and fecundity of individuals, and their importance has been stressed for the evolutionary origins and maintenance of social behaviour in cooperatively breeding vertebrates (Woolfenden & Fitzpatrick 1978; Emlen 1991) and invertebrates (Lin & Michener 1972; Evans 1977; Strassmann & Queller 1989; Wcislo 1997). A growing number of ecological studies has linked group living to constraints in species' biotic and abiotic environments and has found that resource limitation, climate and predation pressure can all play roles in selection for social behaviour. Although the importance of ecological factors has been emphasized for the evolution of social groups, there are few empirical studies tracking the selective pressure imposed by ecological constraints on both solitary individuals and social group fitness in sympatry.

Firstly, depending on species and environment, resources can vary in abundance and ease of acquisition. The basic necessary resources are breeding sites and food, and competition for scarce resources may promote cooperation and group living (Alexander et al. 1991). In insects, cooperative breeding always involves multiple adults raising brood in a central nest (Crespi 1994). When nests are difficult to construct or hard to find then they may become a limiting resource that can be reused from one season to the next. Remaining at the natal nest to inherit such a valuable resource may be a better option than dispersing from the natal nest if chances of independent nest founding are low (Hogendoorn & Leys 1993; Schwarz et al. 2005; but see Bull & Schwarz 1996).

Secondly, natural enemies are important agents of selection in the evolution of group living (Lin 1964; Michener 1985; Uetz & Hieber 1997; Beauchamp 2004). Nesting independently requires a single individual to obtain all brood provisions and therefore there are times when the nest is left unguarded. Guards at the nest provide protection against

attacks on immatures in many social insects (Wcislo et al. 1988; McCorquodale 1989; Sakagami et al. 1990; Matthews 1991; Garofalo et al. 1992), and experimental removal of guards from social colonies leads to lower brood survival in bees (Smith et al. 2003, 2007; Zammit et al. 2008), spider mites (Mori & Saito 2005) and wasps (London & Jeanne 2003).

Thirdly, abiotic factors such as geographic location and local climate are known to have marked effects on life history evolution studies. The effects of variation in climate on social behaviour in bees provide several testable hypotheses. Studies on facultatively social bees, those in which females are totipotent (capable of both solitary and social reproduction) have revealed that some sweat bees are social and produce two broods per year in areas with warmer temperatures and longer breeding seasons but are solitary in areas with cooler temperatures and shorter breeding seasons (Sakagami & Munakata 1972; Packer 1990; Eickwort et al. 1996; Mueller 1996; Hogendoorn & Leys 1997; Soucy 2002; Cronin & Hirata 2003; Brady et al. 2006; Weissel et al. 2006).

The effect of variation in local weather conditions on social behaviour can be as marked as the effect of climate variation on a geographic scale (Sakagami & Hayashida 1968; Packer 1990; Hirata & Higashi 2008; Hogendoorn & Velthuis 1993; Yanega 1993). For example, long term studies of the obligately social sweat bee, *Halictus ligatus* (Richards & Packer 1995) revealed that annual fluctuations in weather conditions influenced rates of brood survival and forms of social organization. Cold, rainy weather reduced the duration of time available for brood rearing, leading to smaller clutch sizes, and also resulted in nest flooding, which led to brood rot resulting in high nest failure and low brood survival rates. Atypically warm weather resulted in an early onset of brood production, larger clutch sizes and, in turn, higher rates of worker oviposition (Richards et al. 1995) as worker numbers and pollen collection exceeded the queen's egg-laying abilities.

No studies to our knowledge have contrasted a socially polymorphic species, with both solitary and social nests in the same population, over a series of brood-rearing periods to investigate how these sources of ecological variation might select for variation in social behaviour. The role of fluctuating environmental conditions has long been considered important for social insects and vertebrates but direct tests have been few (reviewed in Strassmann & Queller 1989; Emlen 1991; Wcislo 1997; Purcell 2010).

Elucidating the environmental conditions that favour either solitary or social nesting strategies requires studying species in which both strategies occur in sympatry, so that the fitness consequences of each nesting strategy can be assessed over a series of brood rearing periods. The Australian small carpenter bee, *Ceratina australensis,* is socially polymorphic (Michener 1962; Rehan et al. 2010), with both solitary and social nests in the same population, thus seasonal and social variation can be compared to examine fitness consequences of solitary and social reproductive strategies. In solitary nests, females forage and reproduce independently while in social colonies, a primary female behaves much like a solitary female, taking on foraging and reproductive duties, while a secondary female remains at the nest as a passive guard and delays reproduction until the next season (Rehan et al. 2010). Females that disperse after eclosion to initiate new nests do so solitarily, however, females that reuse their natal nest may form social colonies. Adult females of this species often survive long enough to be reproductive in two consecutive brood-rearing seasons, either spring then summer, or summer then spring (Rehan et al. 2010). *Ceratina* mothers mass provision brood in a single linear burrow and when oviposition is complete, mothers remain with their nests until the brood reach adulthood (Sakagami & Maeta 1977). This nest loyalty ensures that the contents of complete nests are an appropriate measure of reproductive success because maternal investment and reproductive effort is constrained to a single stem (Rehan & Richards 2010).

The objective of this study is to test predictions of temporal variation in three ecological factors, nest substrate availability, parasitism rates, and local weather as influences on the expression of sociality and the fitness consequences for solitary and social colonies of *C. australensis*. First, nest site limitation should decrease opportunities for females to found nests independently and increase the frequency of social nesting; an increase in nest site availability should decrease the frequency of social colony formation. Second, since solitary bees must leave the nest unattended during foraging bouts and are less able to defend the nest against parasites, we predict increased parasite pressure should increase the fitness and frequency of social colonies. Third, warm dry conditions in the brood rearing season should promote prolonged brood rearing periods and larger clutch sizes. Warmer weather is also expected to accelerate brood maturation; this in turn could favour higher rates of female dispersal and reduce the frequency and fitness of cooperative nesting. On the other hand, cool wet weather is predicted to lower the frequency of female dispersal and limit the brood rearing season which would increase the fitness and frequency of social colonies.

METHODS

In total 982 *Ceratina australensis* nests were collected from dead broken stems of giant fennel (*Ferula communis)* in and around the shire of Warwick in the warm temperate zone of southern Queensland, Australia (28° 13' S 152° 02' E, 480 m elevation). Four collections during brood rearing periods (n = number of nests) were undertaken over a period of 32 months in spring (first week of December) 2007 (n = 145) and 2008 (n = 165), and summer (first week of February) 2009 (n = 241) and 2010 (n = 289).

Nests were collected prior to 7 am to ensure that bees had not commenced flight activity for the day and all occupants would be present. All visible dead, broken fennel twigs with a round hole resembling a bee nest entrance were collected. Twigs were opened by

splitting them lengthwise, and if they contained nests, the contents were recorded, including number of brood cells, brood cell contents, developmental stages of brood, and numbers and locations of adult bees and parasites. Parasites were identified as a single species of chalcid wasp (*Eurytoma* sp.) by Dr. John Huber at the Canadian National Collection of Insects, Arachnids, and Nematodes (CNC) and voucher specimens are retained at the CNC.

Nests were categorized based on contents and overall appearance (Daly 1996, Rehan et al. 2009). 'New' nests had clean walls devoid of pollen stains and faecal pellets while 'reused' nests had darkened walls with pollen and/or faecal stains from previous provisioning and brood rearing in that twig. Complete or 'full brood' nests were those in which the cell closest to the nest entrance contains a larva or pupa, suggesting that the mother had finished laying eggs. Full brood nests were collected at the end of the spring brood rearing season (December) and at the end of the summer rearing period (February). For some analyses, we also included 'active brood nests' which contained pollen masses with eggs or small larvae, and which were deemed not to represent complete broods. 'Clutch size' is the total number of brood cells provisioned in a full brood nest. 'Live brood' is the total number of brood surviving to adulthood in a full brood nest. The proportions of eggs, larvae and pupae in active and full brood nests were evaluated to compare rates of brood maturation among seasons. Samples with higher proportions of pupae would indicate faster rates of brood development, earlier onsets of brood provisioning, or both.

To assess potential nesting substrate limitation in this population, we increased nest site availability by cutting the tips off a patch of 186 fennel stems approximately 10m away from an unaltered patch with existing bee nests. All stems in the altered patch were trimmed with pruning shears to expose bare pith, required for *Ceratina* to nest in these stems. This altered patch was marked with flagging tape in spring 2008 and surveyed for occupancy in summer 2009. If increasing the nest site availability leads to more frequent occupancy than

in unaltered fennel patches this would suggest that availability of dead broken stems may be limiting in the wild.

Climate data were obtained from the Australian Bureau of Meteorology (http://www.bom.gov.au) records for the weather station in the town of Warwick. *Ceratina australensis* are not active during winter months (Michener 1962) when daily maximum temperatures fall below 25°C and, so we assumed that temperatures of at least 25°C are required for bees to forage. *Ceratina australensis* does not forage when it is raining. Foraging days were defined as days above 25°C with no rainfall. To estimate the duration of suitable weather for bee activity each season, the total number of days above base 25°C was calculated for the brood-rearing periods in spring (October-November) 2007 and 2008 and summer (December-January) 2009 and 2010. Since brood cell provisioning and brood development take less than 55 days (Rehan et al. 2010), weather data were compared for two months prior to nest collections to examine weather experienced by the bees during nest provisioning.

Statistical Analyses

Where measures of reproductive success (clutch size, brood parasitism and brood survival) could not be transformed to fit assumptions of parametric analyses (Conover & Iman 1981) we used Kruskal-Wallis non-parametric ANOVA, Mann-Whitney U tests and Chi-square goodness of fit tests were employed to compare temporal variation in reproductive success using SPSS version 16.0.

RESULTS

Weather variation among brood rearing periods

There was considerable variation in temperature (Fig. 1a) and precipitation (Fig. 1b) accumulation among the four brood rearing periods sampled between 2007 and 2010. The 2007, 2008 and 2009 brood-rearing periods were cool, whereas 2010 was average compared to the 30 year mean for each season (Fig. 1a). The total precipitation accumulation (Fig. 1b) varied among periods, 2007, 2008, and 2009 were average and 2010 was dry compared to the other three periods. Combining temperature and precipitation accumulation for each brood rearing period (Fig. 2), the spring 2007 and 2008 brood rearing periods had ten fewer foraging days than the summer 2009 and 2010 periods. This indicates prolonged foraging opportunities in summer compared to spring brood rearing seasons.

Nest site availability

To examine occupation rates in natural and enhanced patches for this species all dead broken fennel twigs with a round hole resembling a putative bee nest were collected. Of a total of 5332 twigs collected between 2007 and 2010, 982 (18%) contained *Ceratina australensis,* 112 (2%) housed other insects, and 4238 (80%) were unoccupied. There was no significant difference in the proportions of unoccupied stems among collections ($\chi^2_3 = 4.339$, $p = 0.227$).

An ancillary patch of 186 dead fennel stems was cut back to expose bare pith and increase nest substrate availability in spring 2008. The following summer of 2009 (i.e., two months later) these stems were surveyed and 13 (7%) were occupied by *C. australensis*, 2 (1%) were occupied by other insects, and 171 (92%) remained unoccupied. There was no significant difference in the proportion of unoccupied stems between natural (991/1111) and

artificially pruned (171/186) stems collected in summer 2009 (Fisher's exact test, $p = 0.317$). Both passive collections of unaltered patches and artificially increasing nest substrate availability revealed that occupied stems were used predominantly by *C. australensis,* while other insects were uncommon, and most stems remain unoccupied. The abundance of unoccupied stems suggests that nesting substrate is not limited.

Variation in brood development

The relative ages of brood from all active and full brood nests collected suggest differences in the timing of nest initiation, in rates of brood development among seasons, or both. In spring 2007 (the first week of December), 19 active and full brood nests were collected, in which 3% (2/74) of immature brood were eggs, 42% (31/74) were larvae and 55% (41/74) were pupae. Conversely, in spring 2008 (also collected in the first week of December), 35 active and full brood nests contained no eggs, 31% (26/84) of brood were larvae, and 69% (58/84) were pupae. This suggests a slight but non-significant delay in brood development in the spring of 2007 compared to spring 2008 ($\chi^2_3 = 4.744$, $p = 0.09$). In summer 2009 (first week of February), from a total of 108 active and full brood nests, 24% (61/258) of immature brood were eggs, 29% (74/258) were larvae and 48% (123/258) were pupae. In summer 2010 (also the first week of February), 216 active and full brood nests were collected, in which 13% (113/870) of immature brood were eggs, 33% (287/870) were larvae, and 54% (470/870) were pupae. Brood development was significantly delayed in the cool summer of 2009 compared to the average summer of 2010 ($\chi^2_3 = 17.33$, $p = 0.0001$).

Variation in reproductive success in solitary nests

The total number of full-brood social colonies in any collection period was too small to examine temporal variation in reproductive success, therefore data presented in this section are for solitary nests only. The proportion of new versus reused solitary nests did not vary among brood rearing periods ($\chi^2_3 = 12.00$, $p = 0.213$). There were no significant differences in clutch size (Kruskal-Wallis $H = 0.18$, $p = 0.683$), number of brood parasitized (Kruskal-Wallis $H = 0.12$, $p = 0.731$), or the number of live brood (Kruskal-Wallis $H = 1.07$, $p = 0.303$) between new and reused nests.

We assessed temporal variation in brood production (clutch size), brood mortality (proportion of brood lost to parasites) and reproductive success (number of live brood) across the four brood-rearing periods of spring 2007, spring 2008, summer 2009 and summer 2010. Clutch size did not vary significantly among brood-rearing periods (Fig. 3a; Kruskal-Wallis $H = 1.625$, $df = 3$, $p = 0.654$). Conversely, variation in the proportion of brood parasitised among brood-rearing periods was marked (Fig. 3b; Kruskal-Wallis $H = 24.933$, $df = 3$, $p < 0.001$). Non-parametric *post hoc* tests for multiple comparisons between treatments (Sigel and Castellan 1988) revealed that nests from 2008 experienced far less parasitism and 2009 significantly greater parasitism than the other years. Consequently, the number of live brood per nest also differed significantly among brood-rearing periods (Fig. 3c; Kruskal-Wallis $H = 20.008$, $df = 3$, $p < 0.001$). Post hoc tests for multiple comparisons between treatments revealed that average brood survival was higher in 2008. It is noteworthy that the highest proportion of brood parasitism and low brood survival occurred during the cool to average season of summer 2009. In contrast, the lowest proportion of brood lost to parasitism and greatest number of live brood also occurred during a cool average season in spring 2008.

Reproductive success in solitary versus social nests

The overall frequency of social nesting was 12% (47/378 active and full brood nests). This frequency did not vary significantly among brood rearing periods (Fig. 4; $\chi^2_3 = 1.259$, $p = 0.74$) and was independent of the frequency of nest reuse in the population ($\chi^2_3 = 0.017$, $p = 0.9842$). The number of full brood social nests was too small in any sample to examine temporal variation in reproductive success. Social colonies were found predominantly (46/47) in reused stems so the effects of nest reuse on reproductive success of social nests could not be assessed.

Social mothers were no more fecund than solitary mothers (Mann-Whitney $U = 1.96$, $z = 0.755$, $p = 0.451$), and variation in clutch size was no greater in solitary than social colonies (Levene's test $F_{32,275} = 1.262$, $p = 0.262$; Table 1). The proportion of parasitized nests was not significantly different between solitary nests and social colonies ($\chi^2 = 0.29$, $df = 1$, $p = 0.59$). The proportion of parasitized brood was not significantly lower in social colonies (Mann-Whitney $U = 18.99$, $z = 1.04$, $p = 0.298$). Parasites claimed 0-50% of brood cells per social colony but never caused complete mortality of the brood. Parasite severity was greater in solitary nests resulting in total nest failure in 11/277 or 4% of solitary nests, but these proportions were not significantly different (Fisher's exact test, $p = 0.197$). When data from all samples were pooled, the number of live brood was significantly greater in social colonies (Mann-Whitney $U = 15.90$, $z = 1.904$, $p = 0.019$). Solitary nests had higher variance in the number of live brood than social nests (Levene's test $F_{32,275} = 7.833$, $p = 0.005$; Table 1). Taken together these data reveal a general pattern of more variable and lower mean reproductive success in solitary nests than in social colonies (Table 1).

DISCUSSION

We examined the fitness consequences of solitary and group living of *Ceratina australensis* in response to three ecological factors: local weather, nest site limitation and parasite pressure. We found seasonal variation in local weather but, contrary to patterns in some other facultatively social bees, this was not associated with variation in the fitness or frequency of social colonies. Nest sites were not limiting and there was no variation in nest reuse patterns among brood rearing periods. Conversely, parasitism did vary among brood rearing periods and had a marked effect on reproductive success in this bee. Overall, this study revealed that solitary and social colonies produce equivalent clutch sizes, but social colonies produce an overall higher number of live brood because of lower parasitism in multifemale nests.

Given the inherently variable nature of environmental factors over time it is important to account for temporal variation and to estimate measures of fitness, such as brood survival, accordingly. Indeed, we observed a general pattern of more variable and lower mean reproductive success in solitary than in social colonies (Table 1). Our results indicate wide variation in reproductive success of solitary nests over the four brood rearing periods that we sampled. At the same time, variation across a number of key nesting and brood-rearing traits was uneven, suggesting that environmental conditions had uneven impacts on several factors that are important for understanding life-history and social evolution. The predicted influences will be addressed in the following sections where we discuss variation in brood production and social behaviour.

Environmental constraints on development rates and brood production

Warmer temperatures cause faster development in insects (Pruess 1983). Therefore it is not unexpected that there was a significant difference in rates of brood development among brood rearing periods. Warmer and drier periods were associated with a higher proportion of pupae than larvae in brood rearing nests. Advanced brood development could also indicate an earlier onset of brood provisioning in warmer weather as found in studies on sweat bees (Richards & Packer 1995; Cronin & Hirata 2003; Hirata & Higashi 2008). Early onset and prolonged warm temperatures during brood rearing periods hasten brood maturation allowing more time for females to initiate nests independently and promote the dispersal of adult females, thus reducing the frequency of multi-female nesting associations.

Clutch size did not vary among brood rearing periods. This is significant given the marked variation in weather parameters and contrasts with some other bee studies in which warm dry conditions were correlated with increased clutch sizes (Packer et al. 1989a; Packer 1990; Richards & Packer 1995; Cronin & Hirata 2003), increased brood production being generally attributed to prolonged foraging durations in warm dry conditions (Minckley et al. 1994; Richards 2004). In our study, precipitation also had no observed effect on clutch size. Precipitation can have drastic effects on ground-nesting bees, leading to flooding, mould and mortality of brood (Packer & Knerer 1986; Packer et al. 1989b; Packer 1992; Heide 1992; Richards & Packer 1995; Fields 1996). Twig-nesting bees such as *C. australensis* remain sheltered from flooding by their elevated nesting habitats, and apparently suffer no other ill effects as we did not observe any signs of brood rot.

An explanation for the lack of temporal clutch size variation in this study may be that this species is not pollen limited; ceratinine females do not forage for the entire brood rearing season, but instead provision a set number of cells and then sit and protect their brood. Clutch sizes of *C. australensis* are reasonably small with an average of five offspring per nest

(range 1-15). Foraging observations on Japanese congeners indicate that females are capable of provisioning 1.6 brood cells in a single foraging day (Maeta et al. 1997). If *C. australensis* provisions at about the same rate, then females would require as few as 3 to 10 foraging days to provision complete broods. This is in contrast to the 20 to 40 foraging days available per brood rearing season (Fig. 2) and suggests that in *C. australensis* foraging time is not limited by weather. In addition, female carpenter bees lay very large eggs and lay at most a single egg per day (Iwata & Sakagami 1966). Consequently, egg limitation may set an upper limit on clutch size rather than provisioning time or pollen availability (Minckley et al. 1994; Rosenheim 1996).

The lack of temporal clutch size variation is further supported by the fact that ceratinine mothers provide prolonged parental care after foraging to sit and protect their brood for the duration of development, inspect brood cells (Rehan et al. 2009; Rehan & Richards 2010; Rehan et al. 2010) and feed offspring prior to dispersal (Sakagami & Maeta 1977). The consistent clutch sizes found in *C. australensis* (this study) and congeners (Vickruck et al. 2010) may be attributable to the energetic requirements of such egg limitation and prolonged maternal care rather than pollen availability or weather variation (Neukirch 1982; Schmid-Hempel et al. 1985; Cartar 1992).

The effect of brood parasitism on reproductive success

In contrast to the lack of temporal variation in clutch sizes, we found significant variation in rates of brood parasitism among brood rearing periods. *Eurytoma* sp. was the only parasite found in this study. *Eurytoma* are known parasites of *Ceratina (Zadontomerus)* species from the Nearctic (Bugbee 1966; Daly 1967; Vickruck et al. 2010) and *Ceratina (Euceratina) callosa* in the western Palearctic (Grandi 1961). The parasite is thought to enter the stem at the entrance and lay its eggs in a series of consecutive cells (Daly 1967). The life

history of this parasitoid species is unknown but seems synchronous with that of its host. Late stage pupae of both the bee host and its parasite were collected in spring and summer broods, which suggests that the parasite, like its host, is bivoltine in southern Queensland.

There was a marked increase in parasite pressure and decreased brood survival during the cool summer of 2009. Bees forage less frequently but take longer foraging trips in cooler ambient temperatures (Minckley et al. 1994; Rands & Whitney 2008). Prolonged absence of the mother from the nest leaves the brood vulnerable to invasion by parasites and predators even if the total time the bee is absent from the nest does not vary (Goodell 2003). Given the similar rates of brood production under different weather conditions, variance in reproductive success of *C. australensis* may be attributable to changes in parasite pressure (Goodell 2003; Lienhard et al. 2010).

Parasite avoidance is a strong selective factor contributing to the maintenance of social nesting. Parasites can claim up to 90% of brood in solitary bees (Bohart et al. 1960) and some bee aggregations have been completely extirpated by parasites (Batra 1966). Increased abundance of parasites in this population could favour group living in *C. australensis*. Our study revealed decreased brood mortality in social nests suggesting a marked benefit to retaining a secondary female at the nest.

Nest substrate limitation and social nesting

Rates of nest reuse did not vary significantly across the four brood-rearing periods examined; bees in newly founded nests represented the majority (ca. two-thirds) of the population each year. Likewise, there was little variation in the relative frequency of social colonies, which are largely restricted to reused nests in this (this study; Rehan et al. 2010) and other *Ceratina* species (Sakagami & Maeta 1977, 1989; Rehan et al. 2009). Low frequencies of nest reuse consistent across all brood rearing periods may limit the extent to which social

nesting can occur. Since dispersal occurs during the breeding period prior to reproduction (Rehan et al. 2010), high rates of dispersal in one season should limit the ability of these bees to respond to increasing parasite pressures that might make social nesting advantageous in the next. If constraints such as parasitism that may give social nests an advantage are not predictable on the basis of recent or current conditions, then we may in fact not expect much variation in rates of social nesting.

Social polymorphism in *C. australensis* may therefore result from bet-hedging by social nesting bees (Seger & Brockmann 1987; Yanega 1988; Frank & Slatkin 1990). The high frequency of solitary nesting suggests that it is the optimal strategy when parasite pressure is low but social nesting is advantageous when parasite pressure is high. If high rates of parasite pressure are unpredictable in the previous season when dispersal occurs, then a polyphenism of solitary and social behaviour would be maintained over time. When stochastic elements are introduced into fitness models, strategies that lead to higher average numbers of offspring need not necessarily increase in frequency over long periods of time (Gillespie 1977). Rather, natural selection tends to favour both large mean fitness and small variance in fitness (Stearns 2000; Orr 2007). By minimizing variance in reproductive success between reproductive bouts, bet-hedging by social nesters results in lower reproductive success in some periods, but total nest failure will claim fewer social colonies of *C. australensis* and therefore the benefits of reduced variance in reproductive success reduce the cost of lower fitness in any given brood rearing period.

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Table 1: Comparison of reproductive success measures in solitary versus social colonies of *Ceratina australensis* from Warwick, Queensland. Reproductive success values are averaged over full brood nests censused in spring 2007, spring 2008, summer 2009, and summer 2010.

Reproductive success		Solitary (n=277)	Social (n=34)
Clutch size	Range	1-15	2-10
	Arithmetic mean ± S.D.	5.20 ± 2.66	5.32 ± 2.45
Number of Parasitized brood	Range	0-7	0-3
	Arithmetic mean ± S.D.	1.20 ± 1.77	0.68 ± 1.01
Number of Surviving brood	Range	0-15	2-9
	Arithmetic mean ± S.D.	3.77 ± 2.72	4.47 ± 2.02

Figure captions

Figure 1: Weather data from Warwick, Queensland. Day 1 began on October 1 for spring (grey lines) and December 1 for summer (black lines) brood rearing seasons. a) Variation in average summer temperatures as represented by cumulative degree-days above base 25°C during each brood rearing period. Summer 2010 was average compared to the 30 year mean and spring 2007, spring 2008 and summer 2009 experienced cooler temperatures. b) Variation in the amount of rainfall among reproductive seasons. The summer of 2010 was dry relative to the 30 year average and summer of 2009, spring 2007 and spring 2008 experienced greater rainfall.

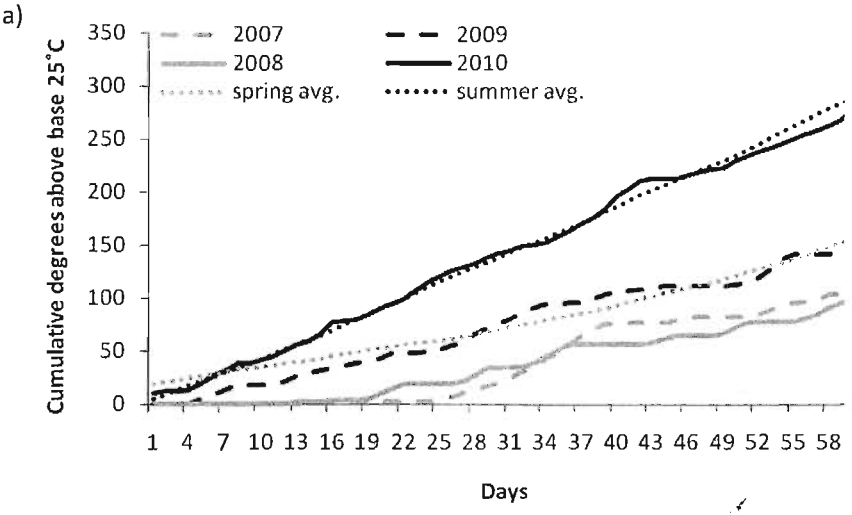
Figure 2: The number of foraging days per brood rearing period. *Ceratina australensis* does not forage below 25°C or when it is raining. The springs of 2007 and 2008 both had fewer foraging days than the summers of 2009 and 2010.

Figure 3: Temporal variation in reproductive success parameters in solitary full brood nests. a) Clutch size, b) proportion of brood parasitized, and c) number of brood surviving to adulthood.

Figure 4: Frequency of social versus solitary colonies among four brood rearing periods. There was no significant difference in the proportion of social colonies per collection. Overall, social colonies represent 12% of reproductive (active and full brood) colonies.

Figure 1:

a)


Cumulative degrees above base 25°C
350
300
250
200
150
100
50
0
2007
2008
spring avg.
2009
2010
summer avg.
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58
Days

b)

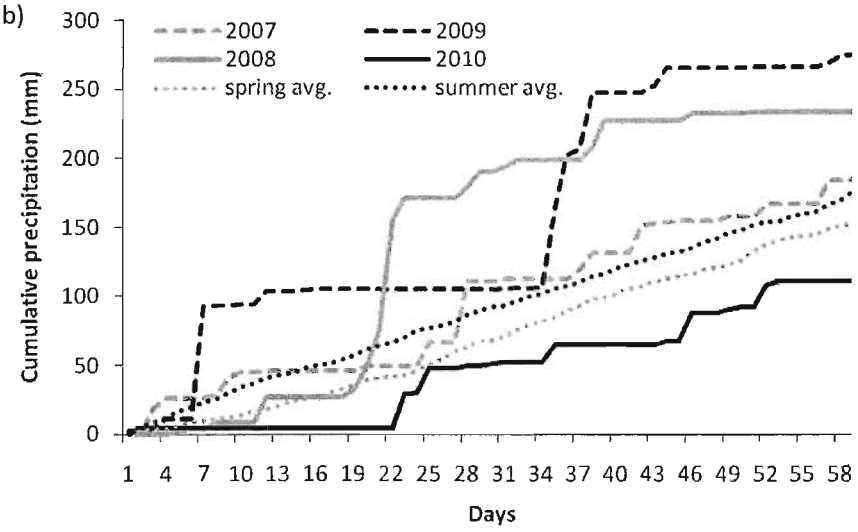

Cumulative precipitation (mm)
300
250
200
150
100
50
0
2007
2008
spring avg.
2009
2010
summer avg.
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58
Days

Figure 2:

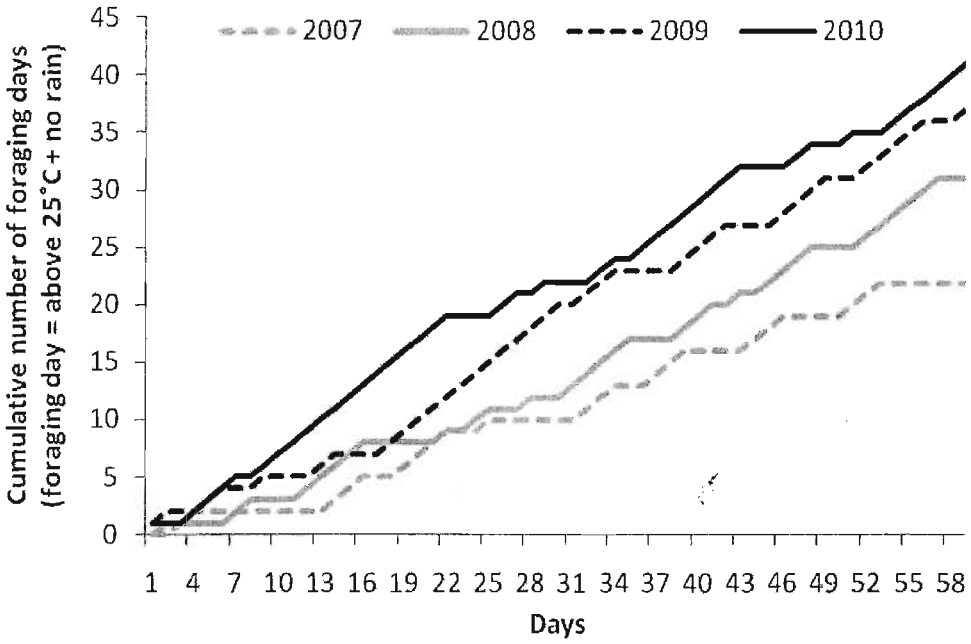

2007
2008
2009
2010
Cumulative number of foraging days
(foraging day = above 25°C + no rain)
0
5
10
15
20
25
30
35
40
45
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58
Days

Figure 3:

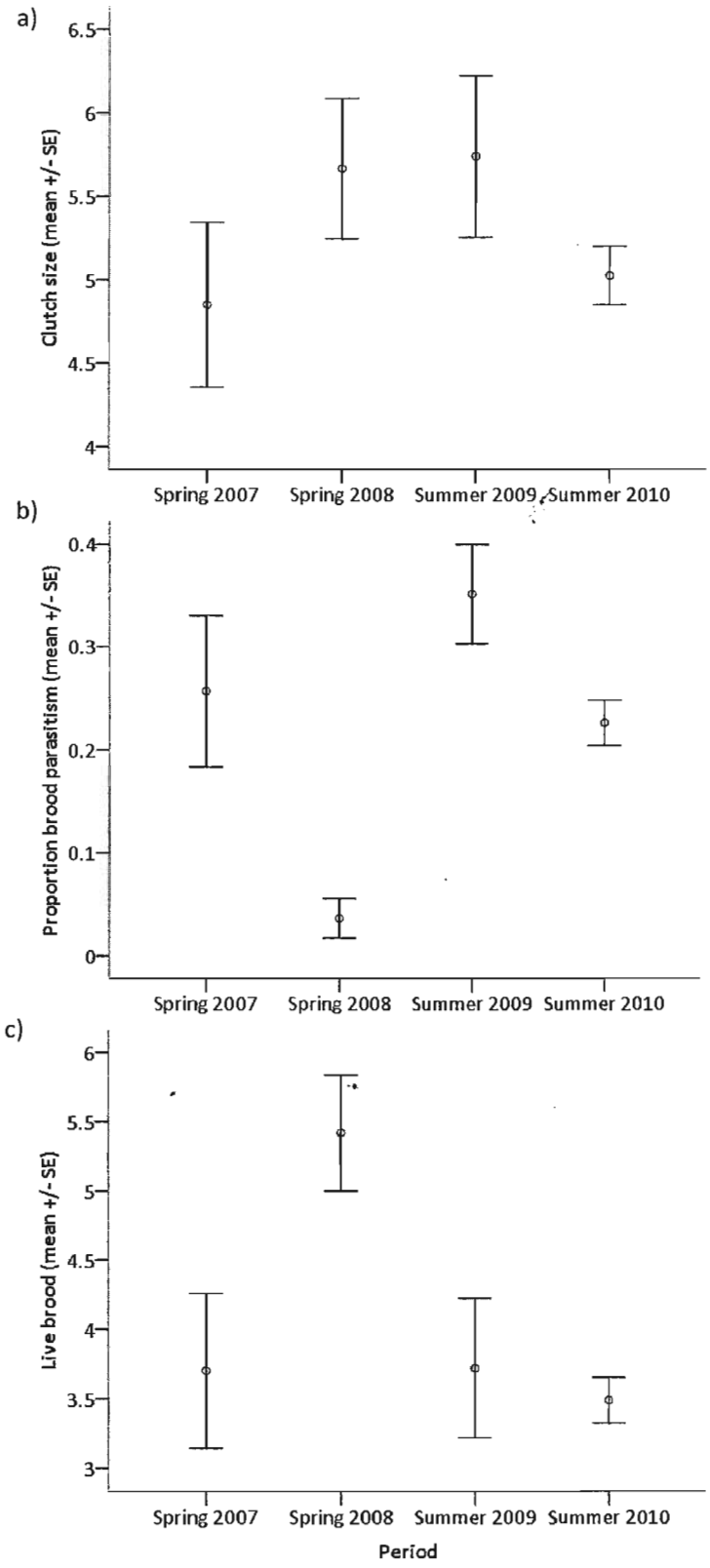
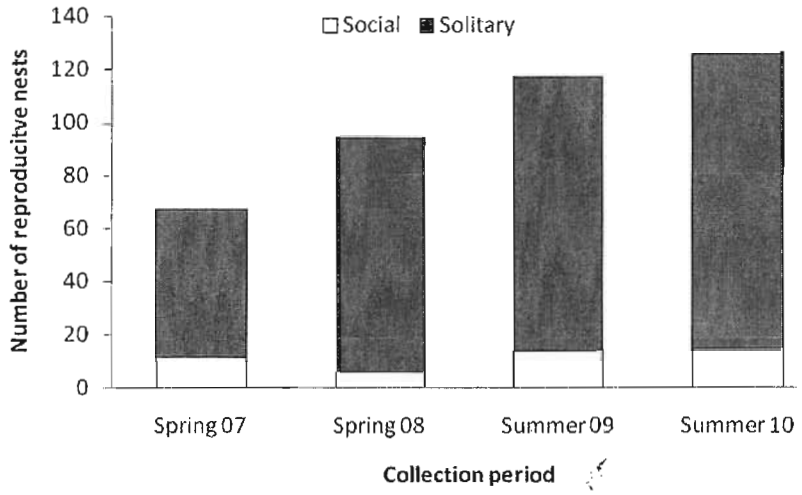

a)
Clutch size (mean +/- SE)
6.5
6
5.5
5
4.5
4
Spring 2007
Spring 2008
Summer 2009
Summer 2010
b)
Proportion brood parasitism (mean +/- SE)
0.4
0.3
0.2
0.1
0
Spring 2007
Spring 2008
Summer 2009
Summer 2010
c)
Live brood (mean +/- SE)
6
5.5
5
4.5
4
3.5
3
Spring 2007
Spring 2008
Summer 2009
Summer 2010
Period

Figure 4:


Social
Solitary
Number of reproducitve nests
140
120
100
80
60
40
20
0
Spring 07
Spring 08
Summer 09
Summer 10
Collection period

Chapter 4:

The costs and benefits of sociality in a facultatively social bee

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Introduction

Whether quantified by biodiversity, biomass, or sheer social complexity, eusocial insects are arguably the most abundant and ecologically successful animals on the planet (1). Eusocial insects dominate their ecological niches yet paradoxically, eusociality has evolved relatively few times. Hamilton (2) proposed that since social groups typically consist of related individuals, an altruist could accrue inclusive fitness through helping to propagate alleles identical by descent (IBD), to those in the altruist. Inclusive fitness has been defined as "the effect of one individual's actions on everybody's numbers of offspring ...weighted by the relatedness" (3). According to Hamilton's Rule, for the simplest pairwise comparisons, individuals could sacrifice reproduction and still pass on more genes IBD when $\mathbf{r_k b > r_o c}$, where $\mathbf{r_k}$ is the relatedness of the altruist to the recipient's offspring, **b** is the number of related offspring raised to maturity, $\mathbf{r_o}$ is the relatedness of an individual to its own offspring, and **c** is the number of offspring the altruist sacrifices by helping.

One mechanism that potential altruists can use to direct their help towards rearing non-descendent kin is to simply become alloparents in their natal nest. This behaviour has the merit of avoiding the costs of risky dispersal, while taking advantage of reproductive opportunities at home. If relatedness is high, the inclusive fitness benefits accrued by remaining at the natal nest to rear a close relative's offspring could outweigh the cost of forfeited reproduction or failed independent nesting. If the number of related offspring raised is high then the inclusive fitness benefits accrued by remaining at the natal nest to co-operatively rear a relative's offspring could be greater than the cost of forgoing or failing reproduction. Likewise, if the number of offspring sacrificed by an altruist is low, the inclusive fitness of helping might be greater than that obtained by reproducing directly.

When comparing social to solitary modes of reproduction, the major question usually asked is why do helpers help? In other words, why would an individual forgo reproduction in

order to aid the reproduction of others? This focal question has also been termed 'the paradox of altruism' and is vital to our understanding of the evolution of social life. The prominence of this focus however has neglected the corollary question: why do individuals accept help? There is a common assumption that accepting help always leads to a net benefit, but there is evidence that group living is costly and helpers may actually have detrimental effects on the fitness of those they help. Possibilities here include workers that do not work, are not as beneficial as they could be, or are detrimental to colony productivity. In paper wasps there are diminishing per capita fitness returns in larger colony sizes, with declining ergonomic efficiency if there are more workers than tasks to be performed (4). Halictid bee workers often reproduce selfishly, lowering the maximum potential fitness of queens (5). In carpenter bees, solitary females sometimes experience higher fitness than dominant females with helpers (6, 7). Therefore it is also important to ask whether dominant individuals should accept help and whether they actually do benefit from having helpers at the nest.

Despite the prominence of theory on the evolution of eusociality and the applicability of mathematical models to answer these questions, direct tests of inclusive fitness theory in social insect populations are few (e.g. 8-11). Previous attempts to evaluate Hamilton's Rule in social insects mostly involve obligately social species, where the question being asked is whether any particular individual should act as a selfish reproductive or a helpful subordinate, but always within a social context. For example, studies on obligately social paper wasps found that sociality is favoured because multiple (pleometrotic) foundresses had higher inclusive fitness than single (haplometrotic) foundresses (8-10). In these studies, direct and indirect fitness were assigned to multiple foundresses but only direct fitness was assigned to single females in their first reproductive effort. These seminal works demonstrated a net benefit to sociality in obligately social wasps and offer insights into the maintenance and elaboration of obligate social groups. However, they do not consider the lifetime

reproductive success of each strategy nor do they provide a genuine comparison of social versus solitary nesting strategies.

A study on obligately eusocial sweat bees also contrasted **r, b** and **c,** comparing reproductive cost to reproductive output for queens and workers and investigated reproductive choices between helping and selfish behaviour (11). This study suggested that helping behaviour does not favour workers, but does favour queens, suggesting that social nesting can be advantageous because of inclusive fitness benefits for some individuals, but not others. However, such a finding still does not address the issue of why individuals should nest in groups versus nesting alone.

To date, only one study on social insects has actually attempted to compare lifetime fitness for individuals breeding solitarily and in groups. Stark (6) evaluated **r, b** and **c** in the carpenter bee, *Xylocopa sulcatipes*. This species is facultatively social, forming both solitary and two-female social colonies; in social nests the inclusive fitness of both solitary nesters and helpers varied over time in ways that could maintain both strategies. Moreover, delayed direct fitness via nest inheritance was an important benefit to being a helper. Unfortunately, this study did not address the fitness consequences for the dominant females in social nests of having a helper, and thus provides no insight into why a dominant bee should tolerate a subordinate in the first place, especially given the risks of nest usurpation and oophagy by nestmates (6).

The basic problem of the evolution of sociality from solitary antecedents is to define the lifetime fitness of each strategy to determine how they would spread through a population. There is a need to define the lifetime fitness of each strategy in as many environments as possible because environmental factors vary. Even within populations, ecological circumstances may have marked effects on the costs and benefits of cooperation. For example, predator or parasite pressure could favour group-living because of possibilities

for joint defence and sustained anti-predator vigilance (12-14). Facultatively social species provide an ideal situation to study the selective advantages of solitary versus social reproduction because females are totipotent and capable of acting as solitary, social reproductive or social helper. This means one can test the functions of inclusive fitness models (r, b and c) maintaining a constant environment while controlling for behavioural trajectories (solitary versus social). More analyses in this vein are critical for proper evaluation of how Hamilton's rule and inclusive fitness applies to the initial stages of social evolution.

Ceratina australensis is a facultatively social bee with both solitary nests (87%) and social colonies (13%) in the same population (15). Social nests consist of only two individuals. Importantly, social colonies exhibit high reproductive skew in which the dominant female forages and lays eggs, while the subordinate female guards and does not forage or lay eggs. Unlike egalitarian (communal) societies where it may be difficult to account for the parentage and helping effort of each group member and offspring in a colony, *C. australensis* is hierarchical, dividing reproduction and foraging effort unambiguously, and it is therefore straightforward to measure **r, b,** and **c**.

Our study tests two related hypotheses. First, females should nest co-operatively when the direct fitness of social nesting exceeds that of solitary nesting. By contrast, females should assume a solitary lifestyle when direct fitness is greater for solitary nesting. Second, when nesting co-operatively, both primary (reproductive) and secondary (non-reproductive) females should have greater inclusive fitness than solitary reproductives. However, if solitary females have equal or greater per capita lifetime inclusive fitness as social females then perhaps different ecological circumstances can explain this behaviour more accurately because **b** and **c** vary depending on environmental circumstance.

Methods

Life history

Ceratina australensis are stem-nesting, small carpenter bees endemic to Australia. Adult females are able to reproduce in two consecutive brood-rearing seasons, either spring and then summer, or summer and then spring (interrupted by a period of autumn and winter inactivity; for full description see ref. 16). New nests are founded by solitary females, and social colonies are formed when females remain together in their natal nest (16). Social nests are occupied by only two adult females. The primary female is both the forager and the reproductive, whereas the secondary remains in the nest, neither foraging nor reproducing. However, if the primary female dies, the secondary female commences foraging and oviposition, thereby assuming the role of a solitary female. This means that a social secondary rarely or never contributes eggs to the first brood, but may contribute eggs to the second brood upon the death of the social primary.

Nest collections and brood production

A total of 982 *Ceratina australensis* nests were censused from dead broken stems of giant fennel (*Ferula communis)* in Warwick, Queensland, Australia. Nests were surveyed in winter, spring, and early summer in 2007 and 2008, and in late summer in 2009 and 2010 (16).

We determined clutch size by counting the total number of brood cells in each nest. Offspring mortality was largely due to parasitism (87%; Rehan unpub. data), and consequently offspring mortality rate is a good indicator of parasite pressure in the population. Brood survival was determined by dividing the number of brood that survived to adulthood by the total clutch size of each brood (15). Direct fitness was defined as the

number of brood produced by an adult female that survived to adulthood produced by each female. This also defined the lifetime reproductive success of each reproductive strategy.

Relatedness estimates

Using allozyme electrophoresis, we genotyped 153 bees from 46 nests (33 solitary and 13 social colonies) collected in the February 2009 sample. Bees used for allozyme analysis were killed by freezing at -80°C in individual 1.5 ml microcentrifuge tubes and stored until assay. Electrophoresis was carried out on cellulose acetate gels (Cellogel™) according to the techniques of Richardson et al. (17). Details of allozyme markers employed are listed in Appendix S1.

We used Arlequin 2.001 (18) to test for linkage disequilibrium among loci and Hardy-Weinberg equilibrium at each locus. These tests were based on a subsample of one randomly selected female per nest. Pairwise linkage disequilibrium values for all loci were ranked and the sequential Bonferroni correction (19) was applied to p-values adjusting for multiple comparisons. Inbreeding coefficient was estimated using the computer program Relatedness 4.2 (20), which was also used to calculate relatedness estimates for mother-offspring and social female pairs. Relatedness 4.2 was also used to generate expected distributions of pairwise relatedness values for specific pedigree relationships, based on the observed frequencies of alleles in our study. For each pedigree simulation, one thousand pairwise values were generated. Estimates are reported as regression relatedness and can range from positive to negative values; zero relatedness represents the average relatedness of any two individuals from the sampled population as a whole (20, 21).

Inclusive fitness was calculated using Gadagkar's (22) formula, which suitably accounts for per capita fitness in a two-female social system. Inclusive fitness equals the direct fitness obtained by the number of offspring produced by each female (as detailed

above), multiplied by the coefficient of relatedness to each offspring (r_o), plus the indirect fitness accrued by the number of offspring raised, multiplied by the coefficient of relatedness to indirect offspring (r_k).

Results

Brood productivity

Solitary females produced similar numbers (mean ± SD) of offspring in their first and second broods (first brood: 5.1 ± 2.7 offspring, second brood: 5.1 ± 2.0 offspring; $t = 0.1209$, $df = 219$, $p = 0.9039$). Social primaries also produced similar clutch sizes in their first and second broods (first brood: 5.3 ± 2.7 offspring, second brood: 5.2 ± 3.0; $t = 0.0361$, $df = 23$, $p = 0.9715$). Therefore, clutch sizes for solitary and primary females were averaged for comparison with broods produced by secondary females that inherited a nest. Social secondaries inherited nests in 10/57 (17%) initially social colonies. When secondaries became reproductive, their mean clutch size was 4.5 ± 2.3 offspring. Since first and second brood clutch sizes were not different in social nests, there was no overall difference in clutch size among solitary, primary and secondary females ($F = 1.90$, $df = 2$, $p = 0.9877$; Fig. 1A).

Solitary females raised similar numbers of surviving offspring in their first and second broods (first brood: 3.7 ± 2.7 offspring, second brood: 3.2 ± 2.4 offspring; $t = 0.8898$, $df = 219$, $p = 0.3746$). Likewise, social primaries raised similar numbers of offspring in each brood (first brood: 4.8 ± 2.9 offspring, second brood: 3.7 ± 2.0 offspring; $t = 0.8059$, $df = 23$, $p = 0.4286$). Social secondaries whose primary had died produced 3.5 ± 2.4 surviving offspring. On average, primary females raised more offspring per clutch than either solitary or secondary females ($F = 3.39$, $df = 2$, $p = 0.0452$). Brood survival (84%) was significantly greater in social colonies with both primary and secondary female were present, than in solitary nests and those inherited by the social secondary (72%; Student-Newman-Keuls *post*

hoc test, $p < 0.05$; Fig. 1B). Therefore in subsequent analyses brood survival rates were considered separately for each reproductive strategy.

Relatedness estimates

Eleven of the 47 putative allozyme loci successfully assayed (Appendix S1) were polymorphic and consistent with Mendelian inheritance at single loci. Chi-square tests revealed that the observed allele and genotype frequencies did not differ significantly from the expected allele and genotype frequencies under Hardy-Weinberg equilibrium for any locus ($p > 0.05$ for all comparisons). There was no evidence of linkage disequilibrium among the loci (10 000 permutations per pair of loci; Bonferroni corrected; $p > 0.05$). The inbreeding coefficient jackknifed over loci was not significantly different from zero (F_{IT}=0.009; p=0.074). Visual inspection of genotypes revealed that all colonies were monandrous and monogynous with no signs of multiple mating or 'alien' genotypes within colonies. In Figure 2 we have graphed the expected distributions of four pedigree relationships between two females, namely full sister, mother-daughter, aunt-niece, and unrelated females. The observed relatedness of social females (0.79 ± 0.09; Table 1) closely matched that expected for full sisters (0.75). The 95% confidence intervals were calculated to compare relatedness estimates with the expected regression relatedness (Table 1) for colonies comprised of a singly mated female and her brood (23). Relatedness estimates from the 11 polymorphic allozyme loci were all well within the 95% confidence interval expected for each known association. As a result, the average relatedness of a secondary to a primary's female offspring (r_k) ought to match the expected value of 0.375.

Direct fitness of solitary and social females

To calculate direct fitness benefits of each reproductive strategy, lifetime reproductive success (LRS) calculations were based on average brood production and observed brood survival rates. This method was employed because colony size did not vary significantly, but there was a significant difference in brood survival among reproductive strategies. Solitary females produced 10.4 offspring over their lifetime, 7.5 of which survived to adulthood. In social nests with no nest inheritance by the secondary female, social primaries produced 10.4 offspring in their lifetime, of which 8.7 survived to adulthood, whereas secondary females did not reproduce. In social nests in which the secondary female inherited the nest, primary females produced 5.2 offspring with 4.4 offspring surviving to adulthood in the first brood while secondary females produced 5.2 offspring in the second brood of which 3.7 survive to adulthood (Table 2).

To compare per capita direct fitness benefits of each reproductive strategy, LRS calculations were based on average brood production of two females, since social colonies all had two females. Based on observed clutch sizes and brood survival rates in solitary and social nests, two solitary females nesting separately would have an average lifetime reproductive success of 15.0 brood. In social colonies, a primary and a secondary female together had a total lifetime brood production of 8.7. Two adult females in a colony that was social for the first brood (i.e. primary and secondary nesting together) and then solitary in the second brood (one female died) had a total brood production of 8.1. Given the observed estimates of clutch size and brood survival, solitary females had greater per capita direct lifetime reproductive success than social primaries or social secondaries (Table 2B).

Inclusive fitness of solitary and social colonies

Combining the lifetime reproductive success of each reproductive strategy (Fig. 1) with the genetic relatedness of social sisters and reproductives to their offspring (Table 1), we calculated the inclusive fitness of each lifetime reproductive strategy (sensu Gadagkar ref. 22; Table 2).

First we considered the reproductive potential of adult females in solitary and social colonies, based on clutch size estimates in the absence of brood survival considerations. When nesting solitarily, females produce 10.4 offspring each, 5.2 in the first brood and 5.2 in the second brood (x the coefficient of relatedness to each offspring, $r_o = 0.5$), resulting in an inclusive fitness of 9.1 each (individual fitness of 5.2 + indirect contribution of 3.9 as a result of 10.4 nieces or nephews x the coefficient of relatedness to each niece or nephew, $r_k = 0.375$). After applying this same calculation to females in all reproductive strategies, we found that solitary females had greater inclusive fitness than primary females, secondary females that do not inherit the nest, and secondary females that do inherit a nest (Table 2A).

Next we considered the inclusive fitness of each reproductive strategy given differential survival rates of brood in solitary and social colonies. For the observed inclusive fitness comparisons, solitary clutch sizes were discounted by the empirical brood survival rate of 72% and social clutch sizes were discounted by the observed 84% brood survival (Fig. 1). This also indicated that solitary females had the greatest inclusive fitness in the population compared to primary females, secondary females that do not inherit the nest, and secondary females that do inherit a nest (Table 2B).

Discussion

Social colonies in *Ceratina australensis* form when two sisters remain at their natal nest. We observed that solitary nesters had greater per capita lifetime reproductive success than both primary and secondary social nesters, thus neither direct fitness nor inclusive fitness explain social behaviour in *C. australensis*. Some indirect fitness benefits are accrued by secondary females as a result of remaining to help their sister, the social primary, raise more offspring to adulthood. However, the size of this indirect fitness benefit did not compensate for the reduced direct fitness of secondary females. Both the observed per capita direct lifetime reproductive success and inclusive fitness for social primaries and social secondaries were lower than for solitary females.

One potential criticism of this and other census-based studies is that solitary nests may in fact have been social nests in which one female departed, thereby overestimating the fitness and frequency of solitary nesting in the population. The fitness implications of social females disbanding prior to reproduction and their nests being deemed solitary when in reality they originated as social colonies are shown in Figure S1. If 20-33% of solitary nests were actually social colonies, in which one female departed prior to reproduction, then social reproduction would have greater inclusive fitness than solitary reproduction in this species. However, these rates of abandonment are far higher than those inferred for this species. The observed rate of nest orphanage for *C. australensis* was 3% of all brood-rearing nests (Rehan unpub. data) and 13% of all nests surveyed, including overwintering and adult assemblages (15). These low values of orphanage observed across all nests suggest that once females establish colonies, they are strongly nest loyal and rarely abandon nests upon initiating reproduction in a stem. Our large sample sizes and prolonged collection periods provide assurance that the proportion of false solitary nests is too low in this species to undermine our

findings that for *C. australensis* solitary nesting is on average more advantageous than social nesting.

Implications of dispersal for social potential

Dispersal prior to brood rearing has strong implications for limiting social behaviour as it disbands groups. After eclosion, all *C. australensis* offspring must either disperse to find and construct a new nest or remain at their natal nest and reuse it for an additional season. We found that social colonies of *C. australensis* are comprised of full sisters that remain at the natal nest, while dispersing females almost all become solitary reproductives. Although earlier studies did not provide genetic data, the prevalence of social colonies in reused nests (24-26) suggests that social colonies predominantly arise when females remain in a natal nest rather than joining a new nest. In contrast, North American *Ceratina* species have never been observed reusing nest substrate and do not form social colonies (27-29). *Ceratina flavipes* in Japan disperse and initiate new nests in autumn (30, 31) and only rarely (0.1% of nests collected) form social colonies in the wild (32). Conversely, their Japanese sister species, *C. japonica,* does not disperse prior to overwintering and frequently forms social colonies in reused nests (31%; ref. 32). The latter two species were studied in sympatry, suggesting that local environmental conditions may be far less important in determining the selection for group living than latent genetic differences in any tendency for dispersal.

The actual cost of dispersal in this population remains unknown, but it is likely that a majority of females disperse successfully, since around two thirds of all colonies are newly initiated each season (16). After modelling lifetime reproductive success in harsher environments (Fig. S2A), we inferred that differential survival during dispersal would have marked effects on the reproductive success of solitary individuals. Solitary females disperse and initiate new nests, whereas social females reuse their natal nest. When the survival of

solitary females decreased, the LRS of the solitary strategy also decreased (Table S1), suggesting that when the costs of dispersal are very high, females would do better to remain at home than leave the natal nest in hope of founding a new nest elsewhere. This model suggests that modification of the cost of dispersal would have important fitness consequences for reproductive strategies in this bee. Under conditions of limited nest substrate availability or perhaps high predation rates on dispersing females, solitary nest initiation might well become disadvantageous and thus females who remain at the natal nest to form social colonies would have a selective advantage.

Reproductive success and direct fitness

In addition to the cost of dispersal, the role of brood mortality and the effects of natural enemies at the nest are known to be strong selective agents on the fitness of social versus solitary reproduction (13, 14, 33). In this study, differences in the observed brood parasitism rates resulted in lower brood survival for solitary females. If parasite pressure were to increase brood mortality for solitary nesters from the observed 28% (72% brood survival) to approximately 60% (40% brood survival; Fig. S2B), the LRS of solitary nests would decrease to the point where it would equal the LRS of social females (Table S1A). However, if parasite pressure increased at the same rate for solitary and social colonies, there would be no point at which LRS of social females would exceed solitary LRS.

Ceratina australensis were not observed during the three year study period to experience the level of parasite pressure required for sociality to become a permanent way of life, as social females had lower fitness than solitary reproductives. However, if parasite pressure varied considerably over time and occasionally became so severe that solitary colonies were continuously extirpated, then the frequency of social nesting might increase considerably, as seen in allodapine bees (34, 35).

Cooperative behaviour and indirect fitness

In this study we found no per capita benefit to group living for social colonies. Despite greater brood survival associated with group living (15), per capita brood productivity was greatly reduced as a function of group living for social females. One explanation for the reduced brood production of social colonies is that it simply reflects the ergonomic limitations imposed by nest architecture. *Ceratina* construct a single linear burrow, with no central brood-rearing cavity or side branches in which two females can construct brood cells, provision and lay eggs concurrently. Nest architecture has marked effects on sociality in xylophilous bees. Social nesting is associated with the construction of branched nests in large carpenter bees (genus *Xylocopa;* ref. 36). Twig-nesting sweat bees (genus *Megalopta*) are capable of producing secondary nest tunnels and can access all brood cell chambers to concurrently work on multiple brood cells (37). The omission of brood cells facilitates concurrent provisioning and oviposition and coincides with the ubiquitous sociality found in the allodapine bees (38). In contrast, studies on relictual carpenter bees (genus *Manuelia;* ref. 39) and the small carpenter bees (genus *Ceratina*; refs. 25, 40) suggest that short, linear nests are not conducive to cooperative nesting, and the nest architecture of wasps is also known to constrain colony size and social organization (41, 42).

Conclusions

The data for *Ceratina australensis* do not fit with a classical inclusive fitness approach. Even if subordinate helpers nested eusocially (with their mothers), their inclusive fitness would still be too low for social nesting to be adaptive. The inability of inclusive fitness models to fully explain behaviours in highly eusocial species has been used by Nowak et al. (43) to argue that inclusive fitness is not sufficient to explain sociality, but their contention is that group selection issues involving complex social dependencies are

responsible for this inability. Our data support neither direct fitness nor inclusive fitness explanations for social nesting. If classical inclusive fitness arguments cannot explain sociality in very simple species like *C. australensis*, then we need to find explanations other than those suggested by Nowak et al. (43).

In the absence of direct and kin-selected fitness benefits for social secondaries in *Ceratina australensis*, two additional mechanisms that might explain the occurrence of social groups are helper subfertility and manipulation of subordinate helpers by dominant reproductives. Since secondary females were capable of reproducing in the absence of the social primary, subordinate behaviour did not result from subfertility (44, 45). In other primitively social bees, manipulation of subordinate helpers by dominant reproductives is often attributed to age and size-based social hierarchies (46-48). In this study, we saw no signs of these predictors, as females were full sisters indistinguishable in body size, morphology, or fat body size (16; Rehan unpub. data). Moreover, physical manipulation via antagonistic interactions has never been observed between cohabiting females in *C. australensis* (Rehan unpub. data) nor any other *Ceratina* species studied to date (24, 25, 49).

Ceratina australensis are quite capable of forming social colonies, but this study reveals that doing so is not adaptive. Forced association experiments in other *Ceratina* species indicate that social behaviour can be elicited in normally solitary species (32, 50). Although sociality has led to the great ecological success of some highly eusocial lineages, including the ants, honey bees and termites (1), most Hymenoptera, insects and animals remain solitary. Here we have provided the evidence to demonstrate that even in a facultatively social insect, social organization may be disadvantageous in terms of fitness, a scenario that ought to limit the spread of this trait. In other words, there need not be a series of intervening species on the road from solitary to social evolution (51), but rather the selective environment (52) must determine the adaptive value of evolving behavioural traits.

Acknowledgements

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Table 1: Intra-colony relatedness (*r*) estimates for *Ceratina australensis* based on 11 polymorphic allozyme loci. Expected *r* estimates based on monandrous and monogynous, haplodiploid regression relatedness (23). Mother - offspring, mother - daughter, mother - son, and full sister relatedness estimates taken from solitary mothers and callow offspring. Estimates were calculated using the computer program Relatedness 4.2 (19). N = number of colonies, n = number of individuals.

Class	Relationship	Expected *r*	Observed *r*	N	n
Solitary	Mother - daughter	0.5	0.616 (0.468 - 0.763)	13	59
	Mother - son	1.0	0.824 (0.593 - 1.056)	9	18
	Full sisters	0.75	0.715 (0.587 - 0.843)	14	77
Social	Full sisters	0.75	0.790 (0.696 - 0.885)	13	26

Table 2: Direct and indirect fitness estimates for solitary and social colonies of *Ceratina australensis* based on Gadagkar (22). A) Fitness estimates based on observed clutch sizes over two broods, but assuming no brood mortality in either solitary or social nests. B) Fitness estimates based on observed clutch sizes and accounting for observed rates of brood parasitism in solitary and social nests (28% and 16%, respectively). Note that in order to equalize sample sizes, the solitary nesting strategy represents the combined values of two independent (solitary) reproductives. Social nesting with no inheritance by the secondary female represents the primary and secondary females when the primary does not die and reproduces in both broods. Social nesting with inheritance by the secondary female represents the primary and secondary females' fitness when the primary reproduces in the first brood and the secondary female reproduces in the second brood.

A) Clutch size				
		Solitary nesting		
	Fitness parameter	Solitary	Solitary	Total brood production
	Number of offspring	10.4	10.4	20.8
	Direct individual fitness	5.2	5.2	
	Indirect fitness	3.9	3.9	
	Inclusive fitness	9.1	9.1	
		Social nesting, no nest inheritance		
	Fitness parameter	Primary	Secondary	Total brood production
	Number of offspring	10.4	0	10.4
	Direct individual fitness	5.2	0	
	Indirect fitness	0	3.9	
	Inclusive fitness	5.2	3.9	
		Social nesting, secondary inherits nest		
	Fitness parameter	Primary	Secondary	Total brood production
	Number of offspring	5.2	5.2	10.4
	Direct individual fitness	2.6	2.6	
	Indirect fitness	2.0	2.0	
	Inclusive fitness	4.6	4.6	
B) Live brood				
		Solitary nesting		
	Fitness parameter	Solitary	Solitary	Total brood production
	Number of offspring	7.5	7.5	15.0
	Direct individual fitness	3.7	3.7	
	Indirect fitness	2.8	2.8	
	Inclusive fitness	6.5	6.5	
		Social nesting, no nest inheritance		
	Fitness parameter	Primary	Secondary	Total brood production
	Number of offspring	8.7	0	8.7
	Direct individual fitness	4.4	0	
	Indirect fitness	0	3.3	
	Inclusive fitness	4.4	3.3	
		Social nesting, secondary inherits nest		
	Fitness parameter	Primary	Secondary	Total brood production
	Number of offspring	4.4	3.7	8.1
	Direct individual fitness	2.2	1.9	
	Indirect fitness	1.4	1.6	
	Inclusive fitness	3.6	3.5	

Figure 1: Rates of brood production and brood survival compared among solitary nesters, social primaries, and social secondaries of *C. australensis*. Different letters above the bars indicate statistical significance among reproductive strategies. A) All strategies produced equivalent clutch sizes in their first and second broods, and there was no significant difference in clutch size among reproductive strategies. B) Primary females had a significantly greater number of live brood than solitary or secondary females.

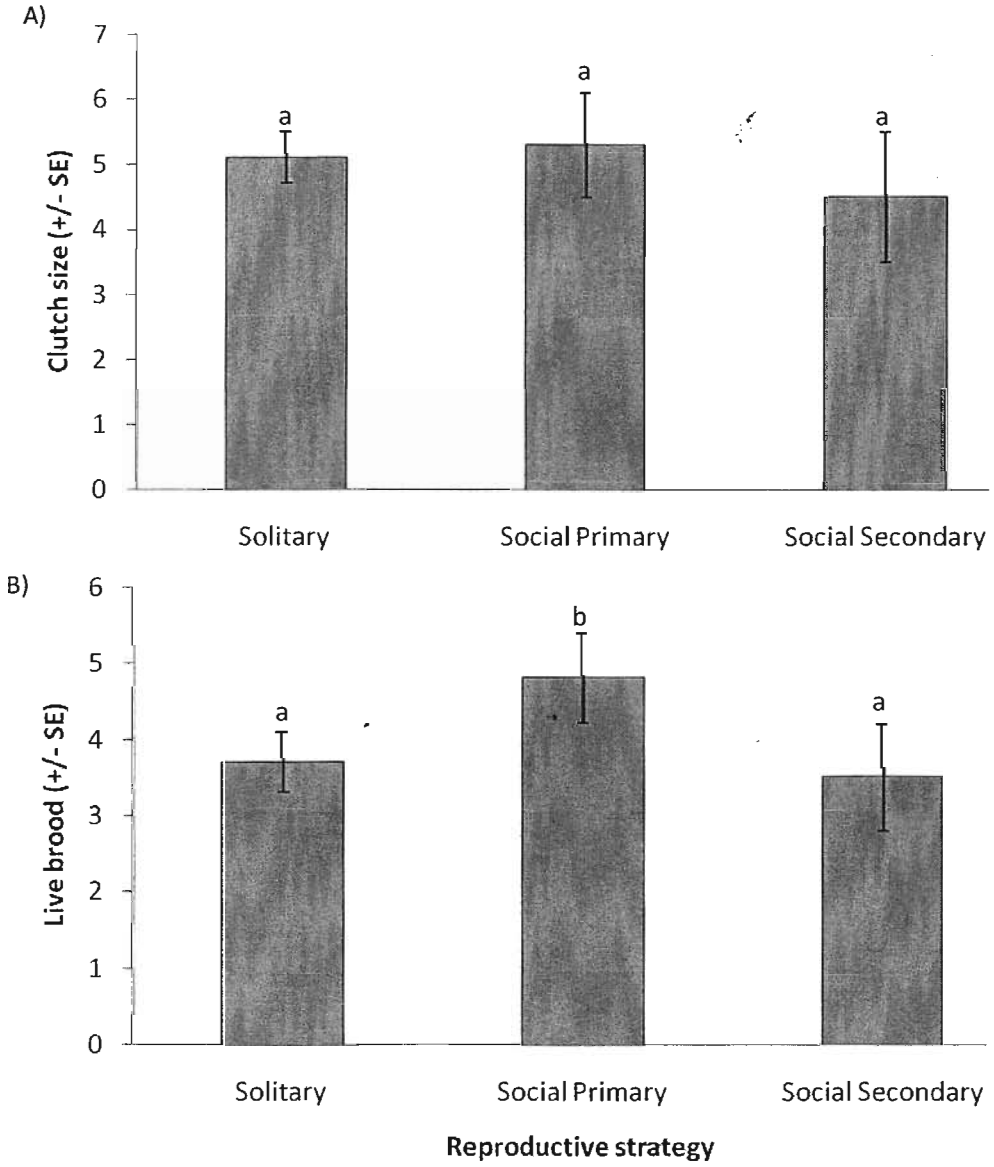
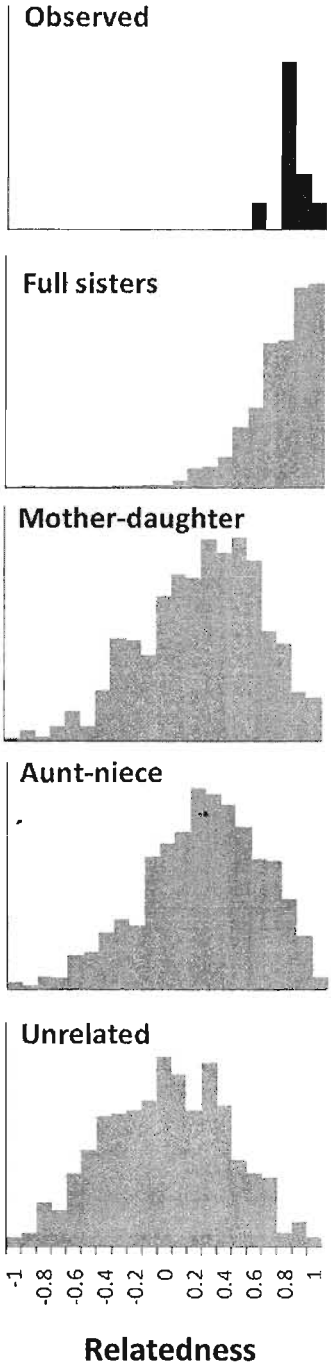


Figure 2: Intra-colony relatedness observed between social females and expected based on haplodiploid relatedness for putative two female relationships. The top histogram (observed social primary to social secondary relatedness, black bars) is for calculated pair-wise values using 11 loci and 13 colonies. Remaining histograms (grey bars) are simulated values based on the same number of alleles and allele frequencies as for the empirical data, but specific pedigree relationships.



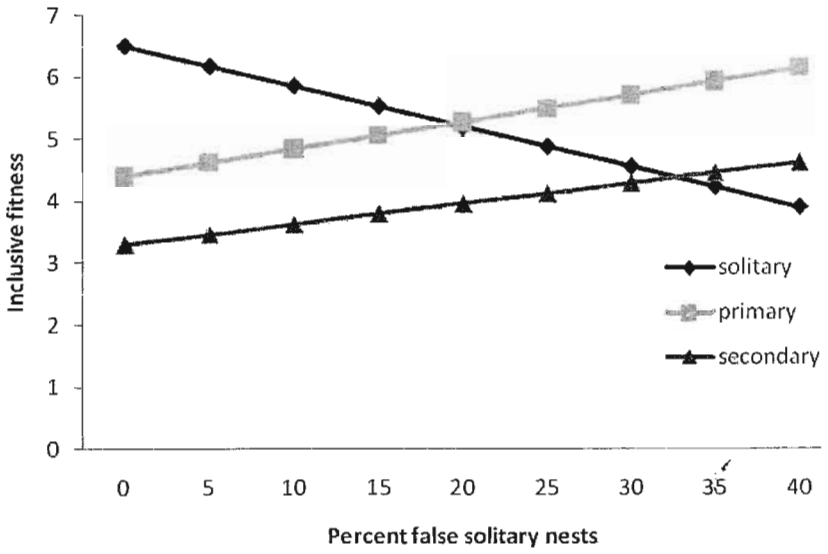
Supplementary Material

Appendix S1: Details of allozyme markers employed. The following 48 enzymes were successfully assayed for the presence of polymorphism: aconitase hydratase (ACON1 and ACON2, EC 4.2.1.3), acid phosphatase (ACP, EC 3.1.3.2), aminoacylase (ACYC, EC 3.5.1.14), alcohol dehydrogenase (ADH, EC 1.1.1.1), adenosine kinase (AK 1 and AK2, EC 2.7.1.20), fructose-bisphosphate aldolase (ALD, EC 4.1.2.13), arginine kinase (ARGK, EC 2.7.3.3), diaphorase (DIA, EC 1.6.99), enolase (ENOL, EC 4.2.1.11), esterase (EST1, EST2, EST3, and EST4, EC 3.1.1.), fumarate hydratase (FUM, EC 4.2.1.2), glyceraldehyde-3-phosphate dehydrogenase (GAPD, EC 1.2.1.12), guanine deaminase (GDA, EC 3.5.4.3), lactoylglutathione lyase (GLO, EC 4.4.1.5), aspartate aminotransferase (GOT, EC 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), glycerol-3-phosphate dehydrogenase (GPD 1 and GPD2, EC 1.1.1.8), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), alanine aminotransferase (GPT, EC 2.6.1.2), hexosaminidase (HEX, EC 3.2.1.30), hexokinase (HK 1 and HK2, EC 2.7.1.1), isocitrate dehydrogenase (IDH, EC 1.1.1.42), cytosol minopeptidase (LAP, EC 3.4.11.1), L-lactate dehydrogenase (LDH, EC 1.1.1.27), malate dehydrogenase (MDH 1 and MDH2, EC 1.1.1.37), malic enzyme (ME, EC 1.1.1.40), nucleoside-diphosphate kinase (NDPK, EC 2.7.4.6), dipeptidase (PEPA1 and PEPA2, EC 3.4.13.), tripeptide aminopeptidase (PEPB, EC 3.4.11), proline dipeptidase (PEPD1 and PEPD2, EC 3.4.13), phosphoglycerate mutase (PGAM, EC 5.4.2.1), phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), phosphoglycerate kinase (PGK, EC 2.7.2.3), phosphoglucomutase (PGM, EC 5.4.2.2), pyruvate kinase (PK, EC 2.7.1.40), L-iditol dehydrogenase (SORDH, EC 1.1.1.14), triose-phosphate isomerase (TPI, EC 5.3.1.1), and uridine diphosphoglucose pyrophosphorylase (UGPP, EC 1.2.1.8). Eleven loci (*Acon2*, *Est3*, *Gpi*, *Hk2*, *Mdh1*, *PepA1*, *PepA2*, *PepD1*, *PepD2*, *Pgk*, and *Pgm*) were informative for pedigree analysis.

Figure S1: Implications of false solitary nests. The possibility of solitary nests being formerly social colonies in which one bee left could not be empirically measured in this study. The observed inclusive fitness of each reproductive strategy is shown as zero. As the percentage of nests deemed solitary that might potentially have originated as social colonies increases the inclusive fitness of solitary females decreased and social females increases. A) Social colonies with no nest inheritance. If 20% of all social nests were falsely deemed solitary, then being a social primary would have greater inclusive fitness benefits than solitary nesting in the population. If 33% of nests were false solitary nests then social secondaries would have greater inclusive fitness benefits than solitary nesting in the population. B) Social colonies with nest inheritance. If 30% of solitary nests originated as social colonies then both primary and secondary reproductive strategies would have greater inclusive fitness than truly solitary nesting females.

Figure S1:

A)


Inclusive fitness
0
1
2
3
4
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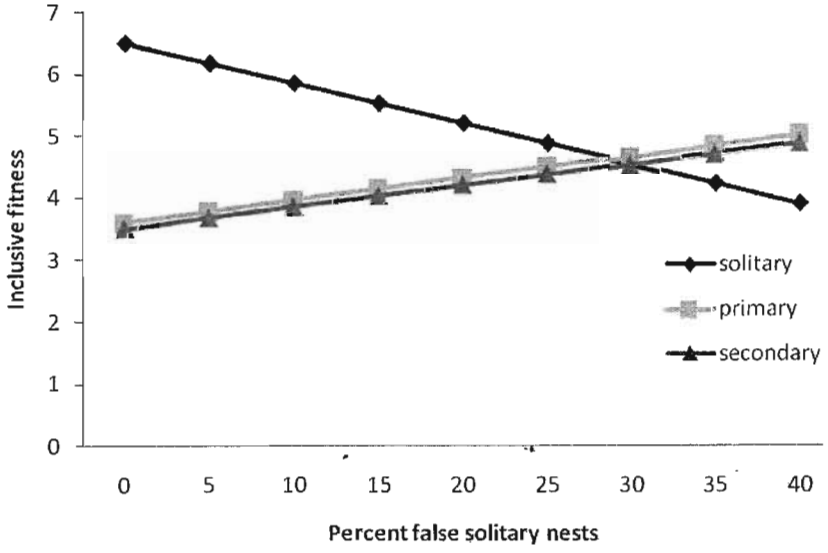
7
0
5
10
15
20
25
30
35
40
Percent false solitary nests
solitary
primary
secondary

B)


Inclusive fitness
0
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25
30
35
40
Percent false solitary nests
solitary
primary
secondary

Lifetime reproductive success formulas

Lifetime reproductive success (LRS) of adult females was calculated from two components: n, the number of eggs laid and *s*, brood survival after parasitism. A female that is solitary when she produces her first brood would also be solitary for her second brood since two female nests only contain sisters, not females of different generations i.e. mother/daughter combinations.

Since average brood production and average brood survival did not differ significantly between first and second brood rearing periods, lifetime reproductive success was calculated based on the overall mean clutch size (n) for all colonies and both broods (n = 5.2). Brood survival rate (s) differed between solitary ($s_{SOL} = 0.72$) and social ($s_{SOC} = 0.84$) colonies (22). For solitary females, LRS was calculated as:

$$LRS_{SOL+SOL} = 2n \times s_{SOL} \qquad \text{[equation 1]}$$

In social colonies the lifetime reproductive success of social ($LRS_{SOC+SOC}$) primary females was calculated as:

$$LRS_{SOC+SOC} = 2n \times s_{SOC} \qquad \text{[equation 2]}$$

where $LRS_{SOC+SOC}$ accounts for the lifetime reproductive success of a social colony in which both primary and secondary females remain in the colony for both the first and second brood production. Lifetime reproductive success of social ($LRS_{SOC+SOL}$) secondary females was calculated as:

$$LRS_{SOC+SOL} = n \times s_{SOC} + n \times s_{SOL} \qquad \text{[equation 3]}$$

where $LRS_{SOC+SOL}$ accounts for the situation in which the primary female monopolizes reproduction of the first brood and the social secondary female remains at the nest as a non-reproductive guard. In brood two the secondary inherits the nest and produces a second brood solitarily if the primary dies. It is important to note that social secondaries were not observed to reproduce in the presence of a social primary. No female that was a social

primary in brood 1, was ever observed to become a social secondary in brood 2. Likewise, no social secondary in brood 1 was observed abandoning the natal nest in brood 2.

Modelled alternative demographic contexts

We modelled the effects of adult and brood mortality to determine the consequences of different ecological circumstances on the fitness of each strategy. In the absence of adult mortality data, we modelled the effect of variation in adult mortality on the reproductive success of each strategy. Since new nests are mostly founded by solitary females, whereas social colonies are usually formed when females remain together in their natal nest (22), we modelled the effect of elevated mortality for solitary females during the dispersal phase of the life cycle. To do this we calculated the decrease in adult survival of a solitary female required for solitary lifetime reproductive success to equal the per capita lifetime reproductive success of females in social colonies. When only half of all solitary foundresses but all social females successfully establish nests, then the lifetime reproductive success of solitary females ($LRS_{SOL+SOL}$) equals the lifetime reproductive success of the social nesting strategy with no nest inheritance ($LRS_{SOC+SOC}$; Fig. S1A). Increasing the mortality rate of dispersing solitary females to 55% resulted in the social nesting strategy with nest inheritance ($LRS_{SOC+SOL}$) having greater reproductive success than two solitary females.

Given the observed different brood mortality rates between solitary and social colonies, we wanted to know what level of brood mortality in solitary colonies would be required for solitary individuals to experience rates of reproductive success comparable to the observed social associations. To achieve this, we varied brood mortality rates of solitary females (s_{SOL}) from 0 to 1 to determine how changes in solitary lifetime reproductive success (LRS_{SOL}) would affect the fitness of the solitary nesting strategy relative to the social nesting strategies (equations 1 to 3; Fig. S1B). Increasing solitary brood mortality rates from the

observed 28% to 60%, an approximate two-fold increase in solitary brood mortality, would result in solitary lifetime reproductive success equal to that of social the social nesting strategy with no nest inheritance. A further increase of solitary brood mortality to 75% would result in social nesting strategy with nest inheritance also having greater lifetime reproductive success than two solitary females.

Modelled alternative inclusive fitness estimates

Similar to the above model on estimates of lifetime reproductive success, we wanted to determine the demographic circumstances that might permit inclusive fitness to select for social behaviour in this species. To do this we modelled the effects of increased brood mortality in solitary colonies (s_{SOL}) on inclusive fitness estimates (Table S1). Using the predicted values from Fig.S1, we saw that increasing brood mortality from the observed 28% (Table 2) to 60% and 75% (Table S1) would result in social nesting with no nest inheritance by the social secondary ($_{SOC+SOC}$) and social nesting with nest inheritance by the social secondary ($_{SOC+SOL}$) reproductive strategies having greater inclusive fitness than solitary females. Once again the observed inclusive fitness estimates select for solitary nesting in this species, however decreased survival of solitary brood could create a selective environment for social behaviour.

Figure S2: Modelled lifetime reproductive success of each reproductive strategy. A) Increasing a cost to dispersal (adult mortality) was predicted to decrease the lifetime reproductive success of solitary nesters ($_{SOL+SOL}$). Note: zero adult mortality equals the observed lifetime reproductive success (28% brood mortality) of each strategy. B) Increased brood mortality in solitary nests was predicted to decrease the reproductive success of solitary reproduction.

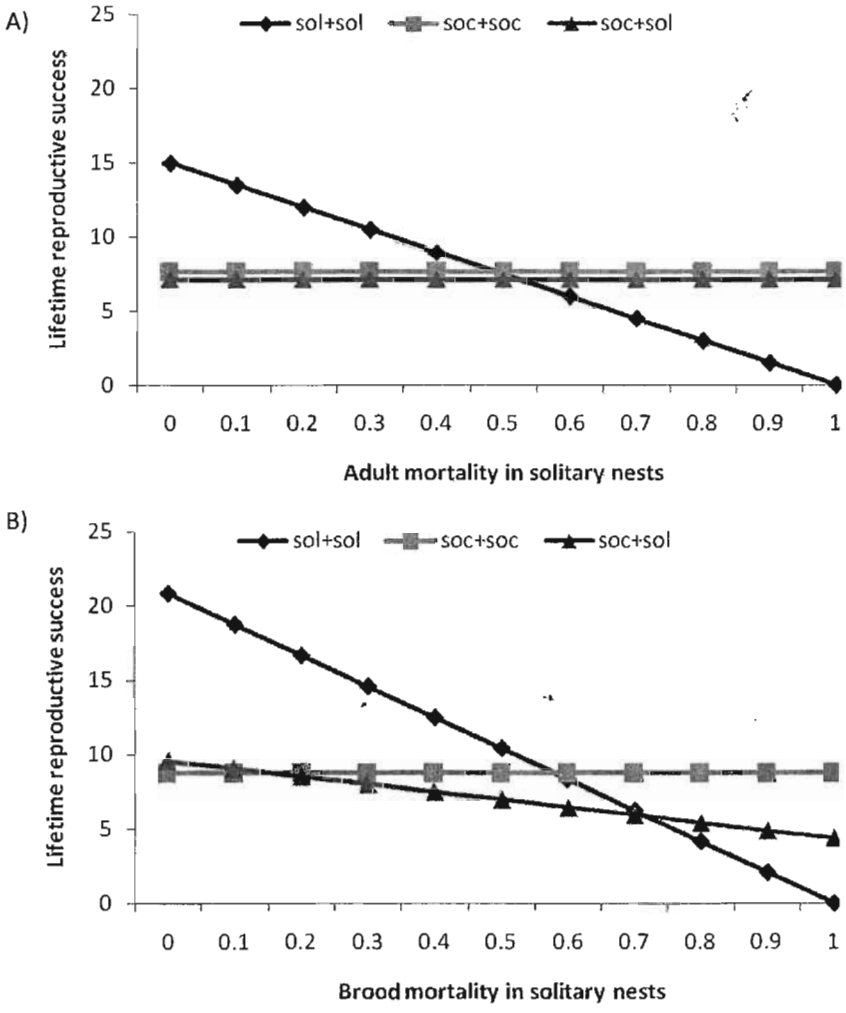

Table S1: Modelled inclusive fitness estimates for solitary and social colonies of *Ceratina australensis*. A) Decreasing the observed brood survival values from the observed 72% to 60% solitary brood survival would select for sterility of social secondaries in the social nesting, even in the absence of nest inheritance. B) Further decreasing solitary brood survival to 25% would select for waiting by social secondaries where nest inheritance occurs, due to their increased fitness compared to the solitary nesting strategy. Note that solitary nesting strategy represents two independent (solitary) reproductives.

A) Decreased solitary brood survival (60%)		Solitary nesting		
	Fitness parameter	Solitary	Solitary	Total brood production
	Number of offspring	4.2	4.2	8.4
	Direct individual fitness	2.1	2.1	
	Indirect fitness	1.6	1.6	
	Inclusive fitness	3.7	3.7	
		Social nesting, no nest inheritance		
	Fitness parameter	Primary	Secondary	Total brood production
	Number of offspring	8.7	0	8.7
	Direct individual fitness	4.4	0	
	Indirect fitness	0	3.3	
	Inclusive fitness	4.4	3.3	
		Social nesting, secondary inherits nest		
	Fitness parameter	Primary	Secondary	Total brood production
	Number of offspring	4.4	2.1	6.5
	Direct individual fitness	2.2	1.0	
	Indirect fitness	0.8	1.6	
	Inclusive fitness	3.0	2.6	
B) Very low solitary brood survival (25%)		Solitary nesting		
	Fitness parameter	Solitary	Solitary	Total brood production
	Number of offspring	2.6	2.6	5.2
	Direct individual fitness	1.3	1.3	
	Indirect fitness	1.0	1.0	
	Inclusive fitness	2.3	2.3	
		Social nesting, no nest inheritance		
	Fitness parameter	Primary	Secondary	Total brood production
	Number of offspring	8.7	0	8.7
	Direct individual fitness	4.4	0	
	Indirect fitness	0	3.3	
	Inclusive fitness	4.4	3.3	
		Social nesting, secondary inherits nest		
	Fitness parameter	Primary	Secondary	Total brood production
				5.5
	Number of offspring	4.4	1.3	
	Direct individual fitness	2.2	0.7	
	Indirect fitness	0.5	1.6	
	Inclusive fitness	2.7	2.3	

Chapter 5:

Evidence of Social Nesting in the *Ceratina* of Borneo (Hymenoptera: Apidae)

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INTRODUCTION

The evolution of eusociality is considered one of the major transitions in evolution (Maynard Smith and Szathamáry1995). In solitary species, offspring disperse and reproduce independently whereas workers in eusocial societies remain at the natal nest and largely forego reproduction to aid the queen in rearing siblings. Socially polymorphic lineages, those containing both solitary and social species, retain the plasticity to allow intra-specific comparisons of solitary with social life. The key to understanding the transition to sociality requires a group of closely related taxa possessing broad social, taxonomic and geographic diversity. Bees provide numerous contrasts to offer insights into the origin of sociality with their range of solitary to social forms.

The small carpenter bees (Xylocopinae: Ceratinini) are commonly regarded as solitary (Michener 1974). All behaviourally classified species share a relatively simple life history. Females disperse from their natal nests and find appropriate nesting substrate. These twig-nesting bees excavate linear burrows in the cores of dead exposed pithy stems. Subsequent to burrow construction, females forage for pollen and nectar provisions that they form into a pollen mass on which they lay an egg. After provisioning and oviposition, brood cells are capped with a partition of wood pith, and the process is repeated in a serial manner along the linear nest chamber.

Some ceratinines exhibit the following four traits unusual in solitary bees (Sakagami and Maeta 1977). First, mothers are nest loyal, ovipositing all of their brood in a single nest and remain at the nest to guard offspring from parasites and predators. Second, mothers exhibit prolonged parental care, periodically inspecting developing brood and incorporating faecal pellets and dead desiccated offspring into brood cell partitions to limit contamination of other developing brood. Third, females are remarkably long-lived, remaining with their developing brood, occasionally surviving to a second reproductive season, and sometimes

even forming subsequent broods. Fourth, mothers and sometimes the eldest daughters, may forage for pollen and nectar to feed newly eclosed brood. Most importantly, multi-female nesting associations in which more than one adult female tends to the brood have been reported in several Japanese temperate *Ceratina (Ceratinidia)* species: *C. flavipes* Smith, *C. japonica* Cockerell, *C. megastigmata* Yasumatsu and Hirashima, and *C. okinawana* Matsumura and Uchida (Sakagami and Maeta 1977; Maeta and Katayama 1978).

Small carpenter bees are found on every continent except Antarctica, with all members classified into a single genus, *Ceratina,* comprising 17 Old World subgenera and six New World subgenera (Michener 2007). Species are most abundant and diverse in the tropical regions in which they are considered to have originated (Iwata 1971). Despite their taxonomic diversity, the social behaviour of most tropical ceratinines remains unknown, although there are descriptions of the nesting biology of *Ceratina (Ceratinula) sp., C. (Zadontomerus) ignara* Cresson (Michener and Eickwort 1966), *C. (Neoceratina) propinqua* Cameron, *C. (Pithitis) smaragdula* Fabricius (Batra 1976), and *C. (Ceratina) dentipes* Friese (Okazaki 1992). Here we present the first account of the life history and nesting biology of four taxonomically described but behaviourally unclassified *Ceratina* from Borneo.

METHODS

Nest Contents

Ceratina nests were collected at six locations in Sarawak, Malaysia (Figure 1) between 8 and 17 August 2007. Broken stems with entrance holes in the exposed pithy ends were collected and the entrance holes sealed with masking tape. Most nests were found in *Mussaenda sp.*, a pink flowering shrub commonly referred to as Bangkok Rose. Nests were

opened the day of collection, upon which their contents, including number and location of adults in the nest, brood developmental stages (Figure 2), presence of parasites, and overall nest appearance, were recorded. Immatures were identified to sex from the pupal stage onward. In addition, elements of the nest architecture were recorded, including nest length, nest width, gallery length, and brood cell septum thickness.

Adult bees were assessed in terms of body size and reproductive status. Head width (HW) was measured across the widest part of the face, including both compound eyes. The proportional size difference between adult females from the same nest was calculated as (larger HW – smaller HW) / larger HW. Wing lengths were measured along the costal vein from the base of the wing to the proximal tip of the stigma. Wing wear scores were used to assess age and foraging effort: unworn bees with no nicks or tears along the apical margins of their forewings received a score of zero, and highly worn bees with completely shredded apical margins received a score of five.

Adult female nest occupants were dissected to determine mating status and ovarian development. Ovarian development was scored as the sum of the lengths of the three largest terminal oocytes (accuracy $\pm$ 0.01mm). Insemination status was determined by the presence of sperm in the spermatheca (the spermatheca of a mated female is opaque, whereas an unmated female has a transparent spermatheca).

Nest Classification

Nests were assigned to categories modified from similar descriptions by Daly (1966), based on their contents and the reproductive status of the adult females found inside. *Founding nests* formed in newly excavated pith are indicated by light interior walls; they are

devoid of faecal pellets or pollen residue. These nests contain adults but do not contain pollen masses or immature brood and are considered to be at a stage prior to pollen mass provisioning, oviposition, and brood cell construction. *Active brood nests* contain one or more pollen masses or immature bees. *Full brood nests* contain brood cells, with the outer-most (youngest) cell containing a larva or pupa. Active and full brood nests with an adult female are termed 'complete' and those without an adult female 'orphaned'. *Mature brood nests* contain adults but do not contain pollen masses or immature brood. Instead, these nests contain callow brood, have darkened interior walls and often contain faecal pellets and pollen residue. These nests are considered to be at a stage between brood development and dispersal.

Statistical Analyses

Descriptive statistics, correlations and one sample t-tests were calculated in SPSS (Statistical Package for the Social Sciences) version 11.0 (SSPS Inc., Chicago). Simulated random sampling was performed with Resampling Stats, version 4.1 for Macintosh (www.statistics.com).

RESULTS

A total of 77 nests containing *Ceratina* species were collected in Borneo, comprising 22 nests of *Ceratina (Ceratinidia) accusator* Cockerell, 32 of *C. (Ceratinidia) nigrolateralis* Cockerell, 19 of *C. (Neoceratina) dentipes* Friese, and four of *C. (Pithitis) smaragdula* Fabricius.

Nest Contents

In mid-August, all four species were reproductive and had nests containing eggs and developing brood (Figure 2). Nests of *C. accusator, C. nigrolateralis*, and *C. dentipes* contained the full spectrum of brood stages from eggs to pupae, whereas *C. smaragdula* nests contained eggs and larvae but no pupae. Nests containing one or more empty brood cells were observed in all species except *C. accusator*. Brood cells with a pollen mass but no egg, were less frequent than empty brood cells (Figure 2).

The total numbers of male versus female pupae of each colony was tallied to estimate the numerical sex ratio (% male) for each species. All three had female-biased numerical sex ratios among pupae: *C. accusator* 11%, *C. nigrolateralis* 19%, and *C. dentipes* 17% (Table 1). None of the four *C. smaragdula* nests contained pupae, so sex ratios could not be calculated. In all species, adult females were larger than males (Table 2). Intraspecific body size, as measured by head width, is more variable in females than males for each species.

Nest Architecture

All four species formed single linear burrows in pithy stems and nest dimensions are summarised in Table 3. *Ceratina nigrolateralis* had especially long nest burrows and left about three quarters of the nest's length as an entrance gallery. Conversely, the three remaining species formed shorter nesting burrows and left galleries approximately half their nest's length.

Given the variability in nest dimensions and nest contents, the correlation between nest length and the number of nest occupants for each species was examined, but longer nests did not house more adult bees than shorter nests (*C. accusator* $r = 0.30$, $n = 15$, $p=0.28$.; *C.*

dentipes r = 0.40, n = 18, p=0.10; *C. nigrolateralis* r = 0.32, n = 29, p=0.09; *C. smaragdula* r = 0.82, n = 4, p=0.18) but the small sample size for *C. smaragdula* entails very low power. There was also no relationship between adult female body size and nest burrow length for any species (*C. accusator* r = 0.17, n = 15, p=0.57; *C. dentipes* r = 0.10, n = 17, p=0.64; *C. nigrolateralis* r = 0.14, n = 29, p=0.44; *C. smaragdula* r = 0.32, n = 3, p=0.80), but again small sample sizes need to be taken into account. In addition, there were no consistent differences between single female and multiple female nest dimensions for each species (Table 3).

Evidence for nest reuse was observed twice. One *C. dentipes* nest (SRI47) (Figure 3) that contained an adult female, had darkened interior walls and an empty, soiled basal chamber 75 mm long capped with a pith septum 2 mm thick. Above the septum there was an egg on a pollen mass in a closed brood cell. A second nest (B18) had a 68 mm long basal chamber capped with a 2 mm pith septum; an adult female *C. nigrolateralis* was found in the antechamber but there was no brood or pollen within the nest.

Colony Structure

The 22 dissected colonies of *C. accusator* comprised seven founding, two active brood, six full brood, and seven mature brood nests. All the founding nests were newly formed burrows with clean pith walls, each containing a single adult female. Both active brood nests also contained a single adult female. Of the six full brood nests, five were complete and one was orphaned, lacking an adult female. Among the seven mature brood nests five were complete and two were orphaned. All complete nests contained only a single adult female.

In total, 19 *C. dentipes* nests were collected, consisting of four founding nests, twelve active brood nests, and three full brood nests. All four founding nests were newly formed burrows with a single adult female. Eleven of the twelve active brood nests contained a single adult female, although one nest (B49) contained two adult females and two brood cells, each with a pollen mass and egg. The smaller female (head width = 1.38 mm, wing length = 1.31mm) was unmated and had very little ovarian development with a score of 0.50 mm. The second female was considerably larger (head width = 1.55 mm, wing length = 1.51 mm), and contained two partially developed eggs (0.99 and 0.65 mm long), as well as one fully developed egg (1.21 mm long). This female was mated and retained nurse cells from recent oviposition, observed as yellow bodies at the pedicel of the ovaries. The proportional size difference between the larger, reproductive female and the smaller, non-reproductive female was 11%. Both females were likely of the same recently emerged generation, as neither had a single nick in her wings. All three full brood nests contained an adult female assumed to be the brood's mother. No mature brood nests were collected for this species.

Of the 32 nests of *C. nigrolateralis*, seven were classified as founding nests, 20 as active brood nests, five as full brood nests and two as mature brood nests. All founding nests contained a single adult female. Six of the seven founding nests were newly formed, whereas one nest (B18) was reused, with dark soiled interior walls. This nest had a basal chamber 68 mm long, capped with a pith septum 2 mm wide. An adult female was found in the antechamber, but no brood or pollen was found within the nest.

Of the 20 active brood nests of *C. nigrolateralis*, 17 were complete, containing a single adult female with her brood; one was orphaned, lacking an adult female; and two were multi-female nests, each containing two adult females. The first multi-female nest (SRI20) (Figure 3) contained two adult females and four brood cells that housed two small larvae and

two eggs. One female was smaller (head width = 2.07 mm, wing length = 1.77 mm) and had three equivalently sized oocytes (~ 0.4 mm each) accumulating to an ovarian score of 1.29 mm. This female had completely unworn wings and was also unmated. The second female was larger (head width = 2.13 mm, wing length = 1.98 mm), mated and had slightly worn wings with a wing wear score of two. This female had one fully developed egg (2.24 mm in length) and two large ooctyes (1.98 and 1.68 mm) and yellow bodies in the pedicel of her ovaries. The proportional size difference between the larger, reproductive female and the smaller, non-reproductive female was 3%.

The second multi-female *C. nigrolateralis* nest (SRI66), contained two adult females and two brood cells, each housing a pollen mass and egg. One female was smaller (head width = 1.87 mm, wing length = 1.83 mm), unmated and had unworn wings. This female had undeveloped ovaries, each oocyte ~ 0.3 mm in length, combining to an ovarian score of 0.99 mm. The second female was larger (head width = 2.13 mm, wing length = 1.85 mm) and had unworn wings. Dissection of this female revealed three partially developed eggs, the largest oocytes 0.67, 1.01, and 0.80 mm in length, summing to an ovarian score of 2.48 mm. This female was mated and had yellow bodies in the pedicel of her ovaries, indicating recent oviposition. The proportional size difference between the larger, reproductive female and the smaller, non-reproductive female was 12%.

Five full brood nests were collected for *C. nigrolateralis*. Four broods were complete with a mother present, and one incomplete, lacking an adult female. Finally, two mature nests were collected. One (B74) contained a wing-worn, mated mother, and one male and five female imagos and the second (B59) contained one wing-worn, mated mother, in addition to one imago of each sex.

Only four nests of *C. smaragdula* were collected, comprising one founding nest and three active brood nests. The founding nest was newly formed, with clean interior walls, and contained a single unmated female. Two of the three active brood nests contained a solitary mother with her developing brood. The third active brood nest (SAR15) (Figure 3) had 2 adult females and at the base of the nest was one capped brood cell containing a pollen mass and egg. The larger female (head width = 2.33 mm, wing length = 2.07 mm) was unmated, had unworn wings, and her three largest oocytes were incompletely developed, each being ~ 0.4 mm in length. The second female was slightly smaller (head width = 2.29 mm, wing length = 1.98 mm), mated, and had unworn wings. She contained three large oocytes, each ~ 0.75 mm in length. The head width difference between the larger, non-reproductive female and the smaller, reproductive female was 10%.

In the absence of behavioural data, reproductive differentiation was assessed by the ovarian score difference among cohabiting females. In each case of multi-female nesting there seemed to be marked differences in ovarian development. To assess this we used a Monte Carlo simulation resampling technique (Sokal and Rohlf 1995). For each species we calculated the mean difference in ovarian score for nestmates in multi-female colonies (Table 4). We then randomly sampled pairs of females from single-female colonies, so that the number of pairs was the same as the number of multi-female colonies in our collections, and then calculated mean difference in ovarian scores for these resampled 'colonies'. This procedure was repeated 1000 times for each species to give a null distribution to determine whether the observed mean difference in ovarian score between nestmates was due to stochastic variation alone. Only 26 of the 1000 simulated mean ovarian size differences were greater than that observed for *C. nigrolateralis* multi-female nests. For *C. dentipes* only 17 of the 1000 simulated ovarian size differences were greater than that observed between

cohabiting females. These simulations suggest that reproductive differentiation in multi-female nests of both species is greater than would be expected from variance among solitary-nesting females. Conversely, in the simulation for *C. smaragdula* over 300 of the 1000 simulated ovarian size differences were greater than that observed in the lone multi-female nest. *Ceratina smaragdula* was the least sampled in the study with only four nests collected in total, so the power of our analyses to detect reproductive differentiation here is very low, and assessment of reproductive differentiation will require further study with larger sample sizes.

Size variation, as measured by mean head width difference, among multi-female nests of each species was explored using the same procedures as above, but none of the simulations suggested that size variation was due to anything more than random variation alone (Table 4).

Maternal Behaviour

When nests were dissected, the locations of adult females and any evidence of guarding or grooming behaviour, including the rearrangement of the pith in the nest, were observed as signs of maternal care. Those females recovered from nests were typically found guarding their brood with their abdomens blocking the nest entrances. Evidence of maternal care was exhibited in three nests. One *C. accusator* nest (B37) contained a single adult female with three pupae, one pink-eyed, one brown-eyed, and one fully pigmented. The pith partitions in this nest were completely loosened, and the mother was found in the second brood cell with the brown-eyed pupa. A second *C. nigrolateralis* nest (SRI57) (Figure 3) contained a single adult female and four offspring ranging in age from full-grown larvae to a white-eyed pupa. Again the pith partitions in this nest were completely disrupted and the

mother was found among loosened pith between her two youngest larvae. These observations show that females remain in their nests during juvenile development and also check on their developing offspring from time to time. Finally, a third *C. nigrolateralis* nest of interest (SRI10) contained a dead adult female who was highly wing worn (5+) and found in the gallery above an empty brood cell and a developing red-eyed pupa. This nest is consistent with the nest loyalty of a *Ceratina* mother who, after completing her nest, stayed with her brood throughout her life and guarded the nest entrance until her death.

To assess the effect of maternal longevity on offspring survival, all active brood, full brood, and mature brood nests were compared for the presence or absence of an adult female. Of 15 *C. accusator* nests, one full brood and two mature brood nests had been orphaned (20%). None of these orphaned broods showed any sign of desiccation or parasitism. Of 27 *C. nigrolateralis* nests, one active brood and one full brood nest were orphaned (7%), and again neither orphaned nest contained dead or parasitized offspring. The remaining 15 *C. dentipes* and 3 *C. smaragdula* nests contained immature brood (active and full brood classes respectively). Among these nests not a single orphaned brood was discovered. However, despite the presence of the maternal guard, 3/15 (20%) of the *C. dentipes* nests were parasitized by a chalcid wasp.

DISCUSSION

Social Organization

The major finding of this study is that *Ceratina* is not a strictly solitary bee. It is noteworthy that none of the multi-female nests appeared to be communal (equally reproductive) assemblages. All active and full brood assemblages containing cohabiting females exhibited reproductive differentiation, with one female mated and the second unmated, suggesting these are semisocial or eusocial nests. It was difficult to assess whether cohabiting females differed in age as these bees had little to no wing wear. Thus, whether nests contained semisocial (adults of the same generation) versus eusocial (mother-daughter) pairs remains unknown. Sakagami and Maeta (1989) examined multi-female nests of *C. okinawana* in relation to adult female body size. The largest head width difference between females was accompanied by greatest reproductive skew. In these eusocial and semisocial associations, the larger female behaved as the guard and primary reproductive, and the smaller female took on a foraging non-reproductive role. When size differences were relatively small, reproductive skew diminished and role reversion of the smaller and larger females took place. Quasisocial nests, where both females are reproductive, were most common between similar sized associations. Size-based reproductive dominance is also recorded for *C. flavipes* (Sakagami and Maeta 1987) and *C. japonica* (Sakagami and Maeta 1984).

In many social species where morphological castes are not present, body size is an important factor contributing to dominance (Batra 1966; Packer 1986; Hogendoorn and Velthuis 1999). Size dimorphism within nesting assemblages of female bees typically suggests reproductive differentiation (Michener 1974). The three multi-female nests belonging to *C. dentipes* and *C. nigrolateralis*, each contained one large female that had a

high degree of ovarian development and was mated, and a smaller female that was non-reproductive and unmated. These data suggest that larger body size contributes to reproductive dominance; smaller females were reproductively subordinate to larger, reproductively dominant females. Conversely, in the single multi-female nest of *C. smaragdula* (SAR15), the larger female had no ovarian development and was unmated, while the smaller female had fully developed ovaries and was mated. However, both *C. smaragdula* females were unworn and a lone egg was found at the base of the nest with newly excavated pith and light interior walls, suggesting that this was a pleometrotic colony resulting from two adult females cofounding rather than reusing a nest burrow.

Body size data are limited for males of this genus but taxonomic records describing both sexes indicate that *Ceratina* species are sexually dimorphic with females consistently larger than males (Van der Vecht 1952; Yasumatsu and Hirashima 1969; Daly 1973; Daly 1988, Rehan and Richards unpub. data). Moreover, the female-biased numerical sex ratios found in this study are consistent with studies on other socially polymorphic ceratinines including newly emerged full broods of *C. megastigmata* which are reported to have a 59.0% female-bias (Katayama and Maeta 1979). The numerical sex ratio (% male) in mature brood populations is also predominantly female-biased in other Old World *Ceratina*: *C. (Neoceratina) australensis* Perkins, 27% (Michener 1962); *C. (Ceratinidia) flavipes,* 37% (Tano 1964) and 32% (Shiokawa 1969); and *C. (Ceratinidia) japonica,* 13% (Shiokawa 1969). Conversely, studies on a persistently solitary New World species, *C. (Zadontomerus) calcarata* Robertson, have reported male-biased numerical sex ratios: 54% (Johnson 1988) and 57% (Rehan and Richards, unpub. data).

Female-biased numerical sex ratios are often associated with sociality in halictine and allodapine bees (Schwarz *et al.* 2007) and are most likely due to local resource enhancement

(deriving from increased per capita brood production in multi-female nests) or production of workers, who do not count as investment in female reproductive function. Evidence for female-biased sex ratios reported here and in other *Ceratina* species is therefore somewhat puzzling given the low frequency of social colonies. Further study is clearly required to quantify this bias, and the possibility of further sex ratio biasing mechanisms, such as partial bivoltinism (Seger 1983) need to be examined. Partial bivoltinism seems particularly promising as a source of bias in *Ceratina* because of the reported adult longevity in some species (Sakagami and Maeta 1977).

Maternal Behaviour

The transition from solitary to eusocial life requires: 1. maternal care, in that mothers must remain at the natal nest in order to interact with their offspring; 2. maternal longevity, so that mothers survive to associate with callow offspring after eclosion; and 3. mutual tolerance, as females must accept one another in the nest in order to coexist and produce successive brood (Lin and Michener 1972; Michener 1985). In general, xylocopine bees are known for their longevity and nest loyalty (Michener 1990). Some *Ceratina* adult females have been observed to live upwards of three years in greenhouse cages and produce three successive broods (Sakagami and Maeta 1977). Maternal longevity is thought to increase brood survival by allowing a guard at the nest entrance to protect the brood from parasitism. Maternal care is also important for newly emerged offspring. Mature brood remain in the natal nest while the mother forages and feeds the offspring via trophallaxis (Sakagami and Maeta 1977).

Evidence of brood cell inspection was exhibited twice: in one *C. accusator* (B37) and one *C. nigrolateralis* (SRI57) nest. Observations of the intranidal behaviour of Japanese species of the subgenus *Ceratinidia* have revealed that mothers periodically enter brood cells and inspect brood for desiccation, incorporating faecal pellets and dead brood into pith partitions (Sakagami and Maeta 1977). Moreover, all behaviourally described *Ceratina* are nest loyal and remain with their mature brood (Rau 1928; Michener 1962; Daly 1966; Sakagami and Laroca 1971; Kislow 1976; Katayama and Maeta 1979; Johnson 1990), even foraging and feeding them (Sakagami and Maeta 1977). The nest loyalty of adult females with their brood allows for interaction with their newly eclosed brood and the persistence of occupants in the natal nest reveals mutual tolerance between mother and juveniles and among siblings.

Social colonies are thought to be selected for due to the benefits of lowering predator and parasite pressure (Lin and Michener 1972; Evans 1977; Andersson 1984). Parasites were found in three of 19 *Ceratina dentipes* nests collected, and in each case a single chalcid pupa was found in a nest attended by an adult female assumed to be the mother of the developing brood. Nest orphaning was low to moderate (0-20%) across species, but did not coincide with parasitism. Hence the presence of an adult female in the nest seems ineffective against these chalcid parasites. Sakagami and Maeta (1977) also found that the presence of mothers provided no protection from fungi or large ichneumonid parasites. However, *C. flavipes* and *C. japonica* nests exhibit 25-50% brood cell mortality when orphaned versus 3-19% when guarded (Sakagami and Maeta 1977) revealing that the presence of a mother at the nest entrance was effective in preventing mortality from small wasp and fly parasites, which were only present in orphaned nests.

Colony Structure

The diversity of brood developmental stages among nests for each species (Figure 2) suggests two possible reproductive patterns. First, females may found nests and provision brood completely asynchronously. Thus, nests in which brood had matured to the pupal and callow adult stages must have been founded earlier than those nests containing eggs and small larvae. A second but not mutually exclusive explanation is that these species are multivoltine. Mature brood assemblages could represent the end of Brood 1, whereas founding and active brood nests could represent the beginning of Brood 2.

Temperate ceratinines tend to emerge in spring and produce a single brood prior to hibernation (univoltine), although they occasionally produce a second brood (bivoltine), and have prolonged developmental times from egg to adulthood, averaging 1.5 to 2 months (reviewed in Sakagami and Laroca 1971). In contrast, subtropical species tend to have multiple reproductive cycles per year and usually mature in less than a month (reviewed in Sakagami and Laroca 1971). Tropical taxa do not experience a quiescent period and are thought to reproduce year round and these species are also reported to have rapid development, maturing from egg to adult in less than a month (Michener and Eickwort 1966). Given the trend of more reproductive bouts and quicker maturation time with decreasing latitude, it is likely that the tropical *Ceratina* described herein are multivoltine (two or more broods per annum). In addition to the longevity and nest loyalty of ceratinine mothers, the ability for brood to mature rapidly allows for the overlap of generations which all contribute to the formation of multi-female nesting associations.

Constraints on Social Nesting

Ceratina nest in linear burrows with a single nest entrance and brood are provisioned one at a time in a serial manner. Thus, the inability of females to concurrently provision and oviposit in their own brood cells might create an impediment to communal nesting. Social nesting is unstable in all *Ceratina* species in which it has been reported; this is probably due to the constant disruption multiple females present each other while provisioning and constructing brood cells within a linear nest.

Multiple female nest occupancy requires females to remain at the natal nest or co-found a new stem. Evidence for nest reuse was observed twice in this study: once in a *C. dentipes* nest (SRI47) and second in a *C. nigrolateralis* nest (B18). Nest reuse is recurrent in Japanese ceratinines. In *C. japonica,* 203 (47%) of 433 nests examined were reused and 63 of these (31.0%) were multi-female nests. Conversely, of the 230 newly built nests, only three (1.3%) contained multi-female associations (Sakagami and Maeta 1984). High rates of multi-female nesting were also recorded for *C. okinawana* as 57/276 reused nests contained multiple females, whereas only 1/133 newly founded nests contained a multi-female association (Sakagami and Maeta 1989). Likewise, in *C. megastigmata,* 4/5 multi-female nests were discovered in reused nests (Katayama and Maeta 1979). These data suggest multi-female nests predominantly arise when females stay in a natal nest rather than joining a new nest.

Further oddities within ceratinine nests arise from the inconsistency of females when provisioning their brood cells. Empty brood cells have been reported in nests of numerous *Ceratina* species (reviewed in Sakagami and Laroca 1971; this study). There are multiple explanations for these empty spaces, including spacers for emerging offspring to pass one another within the linear nest (Malyshev 1913). However, siblings have been observed to

pass over developing siblings without injury in nests lacking empty spaces (Michener and Eickwort 1966; Tano 1964). Observations of trap nesting megachild bees have shown that empty spaces or false cells help to minimize brood mortality due to parasitic wasps (Tepedino *et al.* 1979, Munster-Swendsen and Calabuig 2000). This is a plausible explanation for ceratinines as they are known to have numerous parasites (Daly 1967). Empty spaces have also been interpreted as interruptions in the brood rearing activities (Michener 1962). Observations on *Ceratinidia* species have shown that females only begin foraging and oviposition activities following the formation of the pith septa (Sakagami and Maeta 1987). Given this brood rearing sequence, an interruption such as bad weather or floral resource limitation could result in the formation of a brood cell septum and the omission of pollen provisions. Further physiological constraints such as egg-limitation (Linsley 1958, Rosenheim 1996) could result in a female producing a brood cell containing a pollen provision but no egg. Carpenter bees are known for their disproportionately large eggs compared to other bees (Iwata and Sakagami 1966) and brood cells containing egg-less pollen provisions are recurrent in the ceratinines (Johnson 1988; Rehan and Richards unpub. data; this study). The prevalence of empty brood cells and egg-less pollen masses in this and other studies suggest that parasite pressure as well as egg and resource limitation are pervasive across temperate and tropical *Ceratina* in all behaviourally described subgenera.

CONCLUSIONS

Accumulating evidence from different regions and species all suggest that *Ceratina* are consistently socially polymorphic across Old World taxa. Whether the *Ceratina* of Borneo are semisocial or eusocial remains unknown, however our data strongly suggest that when multiple females nest together, some form of reproductive division of labour occurs.

North American species of the subgenus *Zadontomerus* have been documented as solitary across all aforementioned studies. Conversely, some Old World taxa show recurrent social polymorphism with all behaviourally classified species exhibiting multi-female nesting. Although typically regarded a solitary genus, some Old World *Ceratina* are socially polymorphic; within a population a few females form multi-female nests while the majority of females of the same population remain in a single foundress solitary state. Across all taxa we see recurrent maternal care and longevity, a broad range in adult female body size, and possible parasite avoidance. Prolonged longevity of queens is a prerequisite to the establishment of eusocial life in Hymenoptera (Sakagami and Maeta 1977). Lin and Michener (1972) argued that the amount of size variation among individuals of solitary species was enough to account for the caste-linked size differences found in primitively eusocial species. Furthermore, extrinsic parasite pressure and strong selection to defend a nest may be a driving factor to social nesting (Crespi 1994).

Future studies with larger sample sizes and prolonged study durations are needed to assess each species' behavioural repertoire and life history traits. Moreover, the composite of behavioural data suggest geographic and taxonomic variation in life history traits and social behaviour of the ceratinines, but these findings lack an evolutionary context. A phylogenetic framework is needed to compile and contrast the frequency and circumstance of sociality in these 'solitary' bees.

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Table 1: Population sex ratio (based on pupae) by species. The mean numerical sex ratio over colonies of each species was compared to the expectation of an equal sex ratio using a one-sample t-test for each species (N = number of nests).

Subgenus	Species	N	Females	Males	Total	% Male	P
Ceratinidia	*accusator*	3	8	1	9	11	0.073
	nigrolateralis	11	17	4	21	19	0.002
Neoceratina	*dentipes*	5	5	1	6	17	0.208

Table 2: Head width (mm) of each species by sex.

		Females				Males			
Subgenus	Species	CV (%)	Mean (± SD)	Min-Max	N	CV (%)	Mean (± SD)	Min-Max	N
Ceratinidia	*accusator*	15.7	2.16 (0.34)	1.57-2.97	46	11.6	1.89 (0.22)	1.72-2.20	4
	nigrolateralis	13.0	1.92 (0.25)	1.53-2.73	49	1.9	1.55 (0.03)	1.53-1.57	2
Neoceratina	*dentipes*	5.2	1.54 (0.08)	1.36-1.68	22	n/a	n/a	n/a	0
Pithitis	*smaragdula*	8.2	2.44 (0.20)	2.29-2.77	6	5.5	2.19 (0.12)	2.05-2.33	5

Table 3: Nest architecture measurement data (mm). Mean ± one standard deviation (N = sample size).

		Nest measurements				Brood cell		
Species	Nest class	Length	Width	Gallery	N	Length	Septa Width	N
Ceratinidia accusator	single female	77.8±28.8	3.0±0.0	40.0±4.2	22	5.3±0.5	2±0.0	11
	two female	n/a	n/a	n/a	0	n/a	n/a	0
Ceratinidia nigrolateralis	single female	102.9±49.3	3.3±0.7	76.6±52.8	30	9.4±6.7	2.7±1.1	78
	two female	132.5±13.4	3.3±1.1	111.5±2.1	2	5.3±1.0	1.7±0.5	4
Neoceratina dentipes	single female	64±33.4	2.9±0.3	37.2±21.6	19	7.1±6.8	2±1.4	42
	two female	52	3	33	1	7.5±0.7	1.5±0.7	2
Pithitis smaragdula	single female	81.3±5.2	3.5±0.7	40.0±22.3	4	8.6±1.3	2.9±1.4	11
	two female	74	4	64	1	8	2	1

Table 4: Comparison of the mean observed and expected ovarian score and head width differences (mm) between multi-female nests. Expected differences were generated through Monte Carlo resampling for each species and P is the proportion of simulated differences that were greater than the observed differences, and which can be interpreted as the level of statistical significance.

		Mean Ovarian Score Difference		Mean Head Width Difference	
Subgenus	Species	Observed	P	Observed	P
Ceratinidia	*nigrolateralis*	2.67	0.026	0.159	0.696
Neoceratina	*dentipes*	2.35	0.017	0.168	0.103
Pithitis	*smaragdula*	0.932	0.317	0.037	1.00

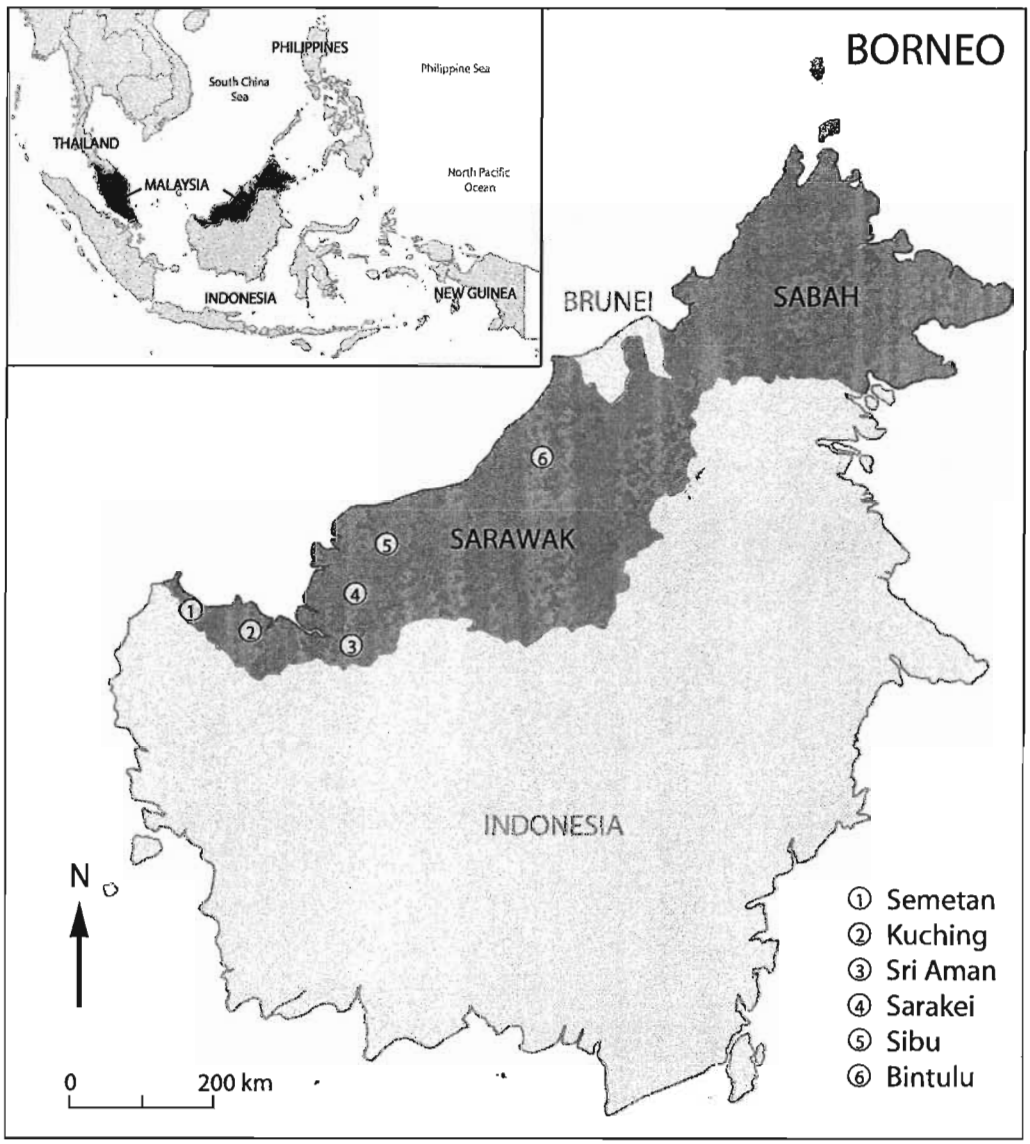
FIGURE CAPTIONS

Figure 1: Map of Sarawak, Malaysia showing *Ceratina* collection locations.

Figure 2: Brood developmental stages of all *Ceratina* nest collections in August 2007. **a)** *C. accusator* brood from 2 active brood and 6 full brood nests. **b)** *C. nigrolateralis* brood from 20 active brood and 5 full brood nests. **c)** *C. dentipes* brood from 12 active brood and 3 full brood nests. **d)** *C. smaragdula* brood from 3 active brood nests. Brood cell provisioning and offspring developmental stages were recorded as follows: unfinished pollen mass in brood cells not forming a complete loaf (*unfpb)*, completed pollen mass without an egg (*pb*), pollen mass with an egg (*pbe*), very small larva 1/3 to 2/3 the length of the pollen mass (*vsl),* small larva 2/3 to 7/8 the length of the pollen mass (*sl)*, medium larva the length of the pollen mass (*ml)*, large larva 1.5 times the length of the pollen mass (*ll)*, full grown larva 2 times the length of the pollen mass (*fgl)*, and prepupa on the verge of pupation with defined head capsule (*pp)*. Pupal stages were recorded based on the darkening pigmentation of their eyes from white to black (*wht, pink, red, brown, blk*), followed by increasing integumental pigmentation from one quarter to full (¼, ½, ¾, *fpig)*. Upon final moult, newly emerged offspring have milky wings (*imago*).

Figure 3: Scale drawings of *Ceratina* nests collected in Sarawak, Malaysia. Each nest represents a different aspect of unusual nest architecture or female behaviour in these species. Two *C. nigrolateralis* nests exhibiting a multi-female nest (SRI20) and maternal nest inspection (SRI57). Two *C. smaragdula* nests showing an empty brood cell (SAR8) and multi-female nesting (SAR15). Two *C. dentipes* nests demonstrating brood cell parasitism (SRI51) and nest reuse (SRI47).

Figure 1:


BORNEO
PHILIPPINES
South China Sea
Philippine Sea
THAILAND
MALAYSIA
North Pacific Ocean
INDONESIA
NEW GUINEA
BRUNEI
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0
200 km
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③ Sri Aman
④ Sarakei
⑤ Sibu
⑥ Bintulu

Figure 2:

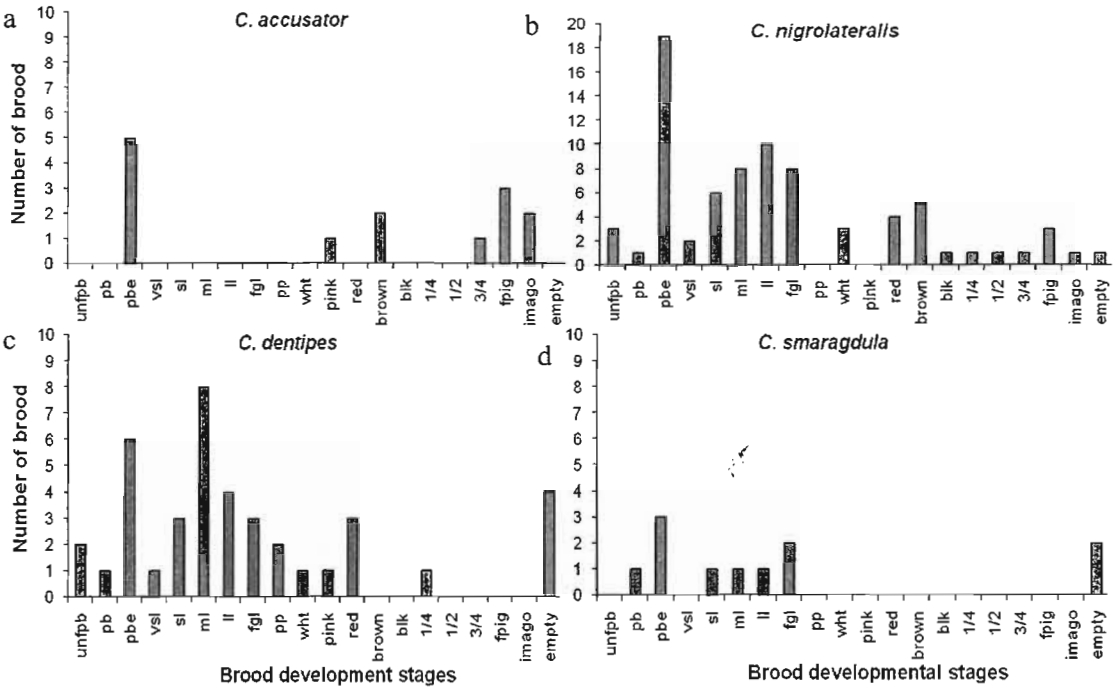
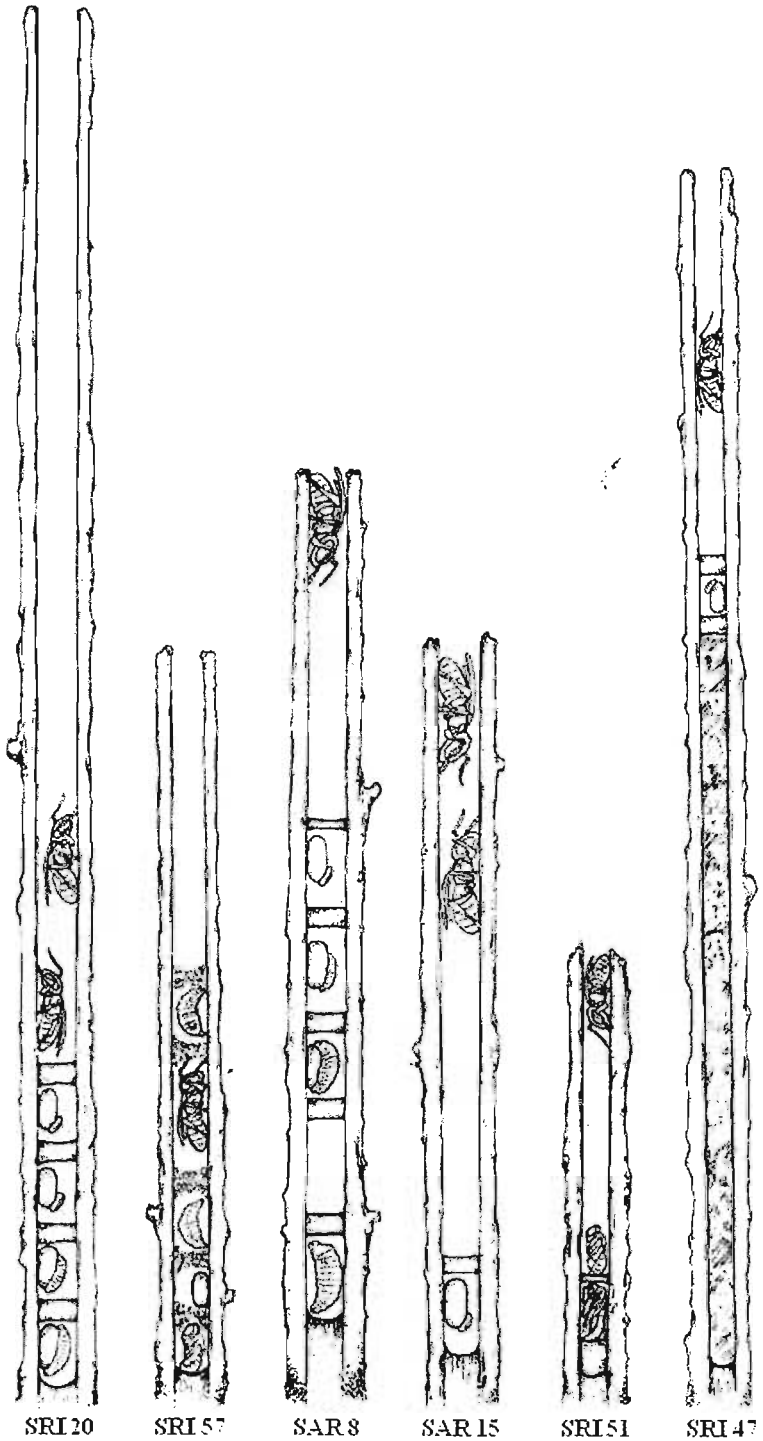

a
C. accusator
b
C. nigrolateralis
c
C. dentipes
d
C. smaragdula
Number of brood
Brood development stages
Brood developmental stages
unfpb
pb
pbe
vsl
sl
ml
ll
fgl
pp
wht
pink
red
brown
blk
1/4
1/2
3/4
fpig
imago
empty

Figure 3:



Chapter 6:

Molecular phylogeny of the small carpenter bees (Hymenoptera: Apidae: Ceratinini) indicates early and rapid global dispersal

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INTRODUCTION

Recent molecular phylogenetic studies of various bee groups are beginning to radically change our understanding of early bee evolution, including identification of the most primitive clades (Danforth et al. 2006), early bifurcations in phylogeny, and some likely biogeographical scenarios for the origins and subsequent spread of bees via dispersal and/or vicariance (e.g. Leys et al. 2002; Schwarz et al. 2006; Hines 2008; Schaefer and Renner 2008).

The first bees probably evolved in the early to mid Cretaceous, corresponding with the rapid diversification of the angiosperms at this time (Grimaldi 1999; Engel 2001; Michener 2007), and this timeframe corresponds with the oldest known bee fossil dated at about 90 million years ago (Mya) and not belonging to any extant family (Poinar and Danforth 2006). There are only two confirmed Cretaceous-age bee fossils, the other being a meliponine bee from New Jersey amber, dated to approximately 65 Mya (Engel 2000). This very limited fossil record means that there are few calibration points when considering the earliest bee divergence dates. However, there are relatively rich fossil bee records from Dominican amber (Miocene) and Baltic amber (Eocene) comprising species from multiple extant tribes, and these have allowed several studies to begin exploring bee phylogeographic and social evolutionary events occurring from the early Eocene until recent times.

A revelation into the origin and evolution of the bees came from the first molecular assessment of the seven extant bee families (Danforth et al. 2006a). Families that were once thought to be relatively derived, including the long tongued bee families Megachilidae, Apidae and Melittidae, now appear to be much more basal. Molecular analysis of the seven bee families coincides with a morphology-based study suggesting a derived origin of the Colletidae along with other short tongued bees (Andrenidae, Halicitidae and Strenotritidae) (Alexander and Michener 1995). The ability to explore evolutionary patterns in bees with

independent data sets has strengthened our understanding, especially when morphology and genetics are congruent (Danforth et al. 2006b). Current diversity and distributions suggest that bees originated in the arid interior of western Gondwana (Michener 1979). Recent molecular phylogenetic data also suggests an African origin as the earliest branches are predominately African lineages (Danforth et al. 2006b).

Molecular studies of the Halictidae suggest an African origin 70 to 55 Mya with subsequent dispersals into South America (70-55 Mya) and North America (55-50 Mya) (Danforth et al. 2004). Molecular studies of allodapine bees (Schwarz et al. 2006; Chenoweth et al. 2007) suggest an African origin for this tribe about 47 Mya, with dispersal from Africa to Australia occurring about 25 Mya, and Fuller et al. (2005) inferred a secondary eastward dispersal from Africa into southern Asia about 18 Mya. Schaefer and Renner (2008) inferred a 56 Mya African origin of the ctenoplectrine bees, with dispersal into Asia 40-30 Mya, from which one lineage reached Australia via Indonesia and New Guinea around 13 Mya. Robust phylogenetic analyses of *Bombus* by Cameron et al. (2007) provided a comprehensive data set to examine their historical biogeography, and using these data Hines (2008) inferred an Asian origin 40 to 25 Mya with subsequent Nearctic and Neotropical dispersal via Bering and Panamanian continental connections around 20 and 7 Mya, respectively. Leys et al. (2002) proposed a Eurasian origin of *Xylocopa* 55-35 Mya with holarctic radiation 34 Mya and subsequent southern dispersal into South America, Africa and Australia < 25 Mya.

The molecular studies of halictids, allodapines, ctenoplectrines, *Xylocopa* and *Bombus* provide insights in terms of current distributions of some bee groups and how those came about. Halictids, *Bombus* and *Xylocopa* all have nearly global distributions (excluding Antarctica, and also excluding the Australasian and sub-Saharan regions for *Bombus*), whereas ctenoplectrines and allodapines both have Old World distributions, with minimal

extension into the Palaearctic for allodapines and minimal austral expansion for ctenoplectrines.

These contrasting distributions raise very interesting questions: do current distributions reflect dispersal ability, times of origin, ecological constraints, or have they been shaped by all three? For example, more global distributions could have arisen from long range dispersal ability *per se*, or it could reflect times of origin that allowed ancestral clades to be dispersed by plate tectonic movements or for dispersal to have occurred over barriers that are large now but were much smaller in the past. The bee tribe Ceratinini (tribe Ceratinini, family Apidae) is the extant sister clade to the Allodapini, but unlike that tribe has a near-global distribution. As such it holds enormous promise for helping to identify factors that may explain differences in geographic distributions among closely related taxa.

The Ceratinini is one of four tribes of the apid subfamily Xylocopinae: Allodapini, Ceratinini, Maneuliini and Xylocopini. To date all studies (Sakagami and Michener 1987; Roig-Alsina and Michener 1993; Engel 2001) agree that ceratinines comprise the extant sister group to the allodapine bees, but while the latter are largely restricted to the southern Old World, with only minimal Palearctic representation, the ceratinines are recorded from all continents except Antarctica (Michener 1979), and the only continent where they are depauperate is Australia (only one recorded species, Michener 1962).

Michener (2007) recognized only one genus in the tribe Ceratinini, containing 21 subgenera, with 16 subgenera endemic to the eastern hemisphere and five endemic to the western hemisphere. Terzo and Rasmont (2007) recently proposed a new subgenus *Dalyatina*, and Eardley and Daly (2007) described eight new species and provided 30 new synonyms in southern Africa without placing many species into subgenera due to a lack of revision of African *Ceratina* species. Some earlier studies accorded generic status to the subgenera *Megaceratina* (Hirashima 1971), *Pithitis* (Klug 1807), and *Ctenoceratina* (Daly

1988) because of their morphological distinctness. However, in a phylogenetic analysis based on morphological characters, Terzo (2000) found that these three latter groups were nested within other clades of *Ceratina* and generic recognition of these groups would render *Ceratina* polyphyletic. Despite extensive effort, Terzo (2000) was unable to definitively resolve the relationships among subgenera based on morphological characters; and therefore the historical biogeography of the Ceratinini has remained largely speculative.

Here we apply molecular phylogenetic techniques to 71 species from 15 ceratinine subgenera to infer phylogenetic relationships, the approximate times of major divergences and the historical biogeography of this tribe. In particular we explore the most likely centre or origin for this tribe, subsequent patterns of dispersal, and what factors may help explain the near-global distribution of the Ceratinini compared to its sister tribe Allodapini.

METHODS

Choice of included taxa

Taxa and sampling localities along with NCBI accession numbers are listed in Table 1. Our ingroup comprised 71 species from 15 of the 21 described subgenera, covering all 6 ecozones of *Ceratina* diversity: Afrotropical (31 species), Madagascar (four species), Indo-Malayan (17 species), Nearctic (four species), Neotropical (five species) and Palearctic (six species). For brevity ingroup species are written using subgeneric names throughout the results as all subgenera and species belong to the genus *Ceratina*. Michener's (2007) subgeneric classification is employed in our study due to a degree of uncertainty of recent subgenera and species groups. Voucher specimens are housed in the collections of M. P. Schwarz at Flinders University of South Australia. In addition to the *Ceratina* species, we included ten species representing all three tribes of the Xylcopinae: Manueliini (two species), Allodapini (seven species) and Xylocopini (one species), as well as two ctenoplectrine, four

corbiculate and two halictine bees to provide fossil calibration points and to help root the ingroup. The allodapines were included because this tribe is the extant sister group to Ceratinini (Sakagami and Michener 1987; Roig-Alsina and Michener 1993; Engel 2001) and, therefore, likely to be most appropriate for rooting the ceratinine clade. The split between Ceratinini and Allodapini also provides a minimum-age calibration point because there is support for a sister relationship between extant allodapines and the Baltic amber fossil tribe Boreallodapini, with the Ceratinini being the next-most basal clade (Engel 2000). *Manuelia* and *Xylocopa* species were also included to sample each of the four tribes and explore the monophyly of the subfamily Xylcopinae. The inclusion of four corbiculate and two ctenoplectrine bees provides another age calibration point between the xylocopines and apines (Schwarz et al. 2006), and two short-tongued halictine bees were included to root this node.

DNA extraction, amplification and sequencing methods

Tissue samples of approximately 5 mg were taken from up to three legs from each specimen. DNA extractions followed Gentra Puregene Cell Kit (Qiagen) standard protocols. PCR amplification was achieved in 20 µl reactions containing 2 µl 10 mM dNTPs (2.5 mM each), 5 µl each primer (5 mM), 1 U HotMaster *Taq* DNA polymerase, 2.5 µl Hot Master *Taq* Buffer ($MgCl_2$ included) and 50 ng DNA template.

Two mitochondrial gene regions and one nuclear gene region were amplified and sequenced bi-directionally. The nuclear exon region was from the F2 copy of elongation factor 1α (EF-1α F2) and the mitochondrial regions were from the protein coding genes cytochrome oxidase I (COI) and cytochrome *b* (Cyt *b*). The primers used for PCR amplification of the EF-1α F2 region included the F2 specific combination HaF2For1/F2-Rev1 (Danforth et al. 1999) to produce an approximately 1100-bp fragment. In the case

where the initial primers failed we used a set of primers designed by S. J. B. Cooper: forward (G1553) 5'-ACTATGTTACCATTATTGACGC-3' and reverse (G1554) 5'-GCTTCTTGCA(G/A)AGC(C/T)TCGTG-3' to amplify a 1060-bp fragment for 36 of the 71 ingroup taxa. Cycle conditions for nuclear DNA were as follows: 94°C, 1 min denaturation; 54°C, 1 min annealing; 72°C, 1 min 30 s extension for a total of 35 cycles (Danforth et al. 1999). The overlapping primer combinations of UEA7/UEA10 (Lunt et al. 1996) and M414/M399 (Schwarz et al. 2004) were used to amplify a 1279-bp COI region when possible. When this failed the COI primer combination of mtd-8 and 12 (Simon et al. 1994; University of British Columbia Biotechnology Laboratory, Vancouver) produced 900-bp PCR product. The Cyt *b* primer combination of cb1/cb2 designed by Y. C. Crozier (Latrobe University, Melbourne, Australia; Schwarz et al., 2004) produced a consistently amplified 428-bp product. Cycle conditions for mtDNA amplification were as follows: 94°C, 1 min denaturation; 50°C, 1 min annealing; 72°C, 1 min 30 s extension for a total of 34 cycles.

PCR products were purified directly using the Multiscreen PCR_{384} Filter Plate (Millipore), and sequenced using 2 µl product in 10 µl reaction volumes for each original PCR primer using the Big Dye Ready Reaction kit Version 3.1 (Applied Biosystems). Sequencing reaction products were then purified by Millipore Filter plate and sent to the Institute of Medical and Veterinary Science (IMVS), Adelaide, Australia for automated sequencing. Forward and reverse sequences were assembled and edited using SeqEd 1.03 (Applied Biosystems). As with the sister tribe Allodapini, the intron regions of EF-1α F2 were largely unalignable at subgeneric and generic levels and were excluded from analyses.

Phylogenetic analyses

Maximum parsimony (MP) analyses were conducted using PAUP* b4.10 (Swofford, 1999) and for Bayesian inference (BI) analyses MRBAYES version 3.1.2 (Huelsenbeck and

Ronquist 2001) was utilized. We relied on BI rather than MP for recovering phylogenies, however, MP analyses were also used to see whether broad topological features were recovered using a very different approach to BI. 100 random sequence stepwise additions were used in the MP analysis, holding 10 trees at each step and with tree bisection and reconnection for searching tree space. Node support was estimated using 500 bootstrap pseudoreplicates, using the same methods as for the heuristic search, and retaining compatible groups with less than 50% bootstrap support.

Molecular analyses of allodapine bees, the extant sister clade to Ceratinini, found substantial problems in resolving phylogenetic relationships using maximum parsimony when 3rd codon positions for mitochondrial genes were given equal weight to other gene partitions (Bull et al. 2003; Schwarz et al. 2003, 2004); thus we explored the effects of down weighting this position between zero and 0.5. This is likely due to the high level of homoplasic changes for mitochondrial nucleotides where AT bias is extreme for 3rd positions (Schwarz et al. 2004). This problem is likely to be at least as problematic where AT composition for 3rd mitochondrial positions in our sample was 82% and where the more basal birfurcations in ceratinines are likely to be older than for allodapines. At the same time, mitochondrial 3rd codon differences are likely to be useful for recent divergences where overwriting is less likely. We used exploratory analyses to examine what kind of weighting for 3rd codon positions minimized the number of equally most-parsimonious trees, and this involved a trade-off between resolution of basal and distal nodes. We settled on a weighting of 0.2 to generate a first topology, and then re-weighted all sites using the re-scaled consistency index implemented in PAUP*.

In the BI analyses the data were partitioned into six parts: 1st, 2nd and 3rd codon positions for the two-mitochondrial genes combined, and 1st, 2nd and 3rd codon positions for EF-1α. All genes were partitioned into three parts due to the varying base composition found

between codon positions. We prefer an 'objective' Bayesian approach (Berger 2006) and therefore used the MrBayes version 3.1.2 default priors because these are mostly uninformative. We used a 6-parameter (Nst = 6) rate transition matrix, with gamma shape for variation in rates and a proportion of invariant sites assumed corresponding to a GTR + I + Γ model. This is the least restrictive model available in MrBayes and allows more restrictive models, such as HKY and K2P which are subsets of the GTR + I + Γ model, to arise if they provide a better fit to the data. All parameters were unlinked between partitions. Two sets of four Monte Carlo Markov Chains (MCMC) with Metropolis Coupling were run in parallel for each BI analysis and convergence was assessed by the average standard deviation of split frequencies and stationarity indicated by plateauing of log likelihood values. The analysis was run for 20 million generations, sampling every 500th generation to reduce auto-correlation among sampled generations and we used a burn-in of four million generations, well after stationarity was reached.

Dating analysis

We used a penalised likelihood method, implemented in r8s version 1.70 (Sanderson 2002) to estimate the ages of key nodes in our phylogeny. We employed three calibration points: (i) the minimum divergence between the Ceratinini and Allodapini was set at 45 Mya because of the fossil Boreallodapini species found in Baltic amber (Engel 2001a). Boreallodapini is the sister tribe to the Allodapini and the Ceratinini is the next most basal clade in the Xylocopinae. This minimum age restriction is likely to be highly conservative since the Allodapini+Boreallodapini clade is likely to have diverged from the Ceratinini much earlier than this. (ii) We also set a minimum age for the node separating *Apis mellifera* from *Liotrigona* B1 because of the fossil meliponine bee *Cretotrigona prisca* recovered from New Jersey amber (Michener & Grimaldi 1988) and most recently dated at 65 Mya (Engel

2000). (iii) Lastly, we set a fixed age of 90 Mya for the node connecting the xylocopine tribes to the corbiculate apines. Fossils of the plant family Clusiaceae, whose floral morphology is closely linked to pollination by corbiculate bees, are dated to 90 Mya (Crepet & Nixon 1998). This node age is also likely to be conservative, so we followed Chenoweth et al. (2007) by exploring the effects of setting this node to 100, 110 and 120 Mya. However, Danforth et al. (2004) have dated the crown age of the Halictidae at approximately 120 Mya, and this family is much more derived than the Apidae, again suggesting that setting the root node at 90 Mya is conservative. The only fossil assigned to the tribe Ceratinini, *Ceratina disrupta* Cockerell (1906), from the Oligocene Florissant shale was not included because the specimen is not confidently placed in this tribe (see Daly 1973).

Because the consensus phylogram had low PP support for several nodes close to the root node of the Ceratinini, any differences between the consensus phylogeny and the actual phylogeny are likely to generate compounding errors when estimating crown ages for descendent clades, even though many of those may have strong support for monophyly. In order to take phylogenetic uncertainty into account when estimating nodes ages we used the following procedure. Firstly, we used MS Excel to randomly select 1000 out of the 24,000 post-burnin phylograms from the MrBayes analysis and we transformed these into chronograms using r8s, with the same smoothing value that was used to generate the chronogram from the consensus phylogram. We then identified a number of internal nodes that had strong PP support ($\geq$ 95%) for monophyly from the MrBayes analysis and used the Most Recent Common Ancestor (MRCA) command in r8s to define these nodes and we then estimated their ages. For each of these nodes we estimated the arithmetic mean age and then sorted the individual estimates, based on the 1000 randomly selected post-burnin generations, in ascending order. For these 1000 sorted age estimates, we then removed the lowest and

highest 25 values, leaving us with a 95% central distribution of ages based on the r8s transformed post-burnin phylograms.

To explore the robustness of our r8s dating analysis we also carried out a relaxed clock Bayesian analysis implemented in BEAST version 1.5.2 (Drummond et al., 2002, 2009). The combined mtDNA dataset and EF1-alpha data set were used with unlinked GTR models of nucleotide substitution, gamma rate heterogeneity and a proportion of invariant sites for different codon positions of mtDNA and EF1-alpha, giving a total of 6 separate partitions. A single relaxed molecular clock using the uncorrelated lognormal model was applied to the entire data set and a constant population coalescent with the Yule Prior was used (Drummond et al., 2002). We used the same calibration points as in the PL analysis, except that instead of setting a minimum age for the MRCA of allodapines and ceratinines we used uniform prior bounded between 45 and 80 Mya, and a uniform prior bounded between 65 and 80 Mya was used for the MRCA of the corbiculates and root of our tree was assigned a normal distribution with a mean of 90 Mya. The analyses were carried out for 20 million generations, sampling every 1000 generations, after which the program Tracer (version 1.4.1) was used with a burnin of 3.5 million generations to check for convergence of the parameter estimates and determine the mean and 95% confidence intervals of the time to MRCA estimates. Time to MRCA estimates along with high probability densities (HPDs) were only obtained for the highly supported clades identified in the MrBayes analysis.

Exploring diversification rates using lineage through time (LTT) plots and Gamma values

LTT plots are frequently used to graphically explore diversification rates, though caution is needed in their interpretation (e.g. Ricklefs 2007). Because our consensus phylogram from the MrBayes analysis had low PP support for some critical nodes close to

the root node (see Results below) we generated a LTT plot for the consensus chronogram as well as for 49 randomly chosen post-burnin chronograms. We used the mltt.plot module in APE (Paradis 2006) to generate 49 LTT plots for the post-burnin samples and superimposed the LTT plot for the consensus chronogram onto these.

The gamma statistic (γ, Pybus and Harvey 2000) is frequently used to quantify changing rates of diversification over time, with lower values indicating greater diversification closer to the root node. However, there are two possible confounding factors that may make interpretation of γ problematic. Firstly, any particular tree topology may not indicate the true branching order of some of the nodes, and if unreliability of nodes varies with time since the root, any single estimate of γ may be biased. Low support for many basal nodes in our results (see below) make this a potential problem. Secondly, our included taxa represent only 71 of the 339 described species in *Ceratina*, and incomplete taxon sampling will tend to produce gamma values that will suggest higher rates of cladogenesis closer to the root (Pybus and Harvey 2000). To explore these possible confounding effects we used the following procedure. We randomly selected 1000 trees from the 24,000 post-burnin trees, subjected these to r8s transformations, and then used TreeEdit (Rambaut and Charleston 2001) to prune all non-ceratinine taxa from the trees. We then used the mccrTest module in Laser 2.2 (Rabosky 2009) to calculate gamma values for these trees. We then used Laser to generate 5000 random trees with a total number of 339 tip species and randomly pruned species to end up with only 71 terminals, and then calculated γ values for these trees.

Biogeographic analysis

We used BayesMultiState implemented in BayesTraits (Pagel et al. 2004; Pagel and Meade 2006) to infer ancestral states and likely vicariance and dispersal events that shaped the current distribution of ceratinines. This method was used because it allows for both

polymorphism in character states (ecozone regions in our analyses) within species as well as uncertainty in phylogeny, which is critical in our analyses where some nodes had low support (see below). Various priors were explored, with a criterion that acceptance rates had to be bounded by 20 and 40% (Pagel and Meade 2006). We used a rate deviation prior of 15 with both an exponential (0.0, 10) reverse jump hyperprior (rjhp), and also explored an exponential (0, 5) rjhp with a rate deviation of 20. The two sets of priors did not give appreciably different results and results from the first set of priors are presented here. Stationarity in the Bayesian run was explored by plotting the harmonic mean and looking for a plateau in this. We subsequently used $40 * 10^6$ iterations with a burnin of $10 * 10^6$, sampling every 1000^{th} generation.

We recorded members of each subgenus as being present in any of seven ecozones: Afrotropical (A), Madagascar (M), Nearctic (N), Neotropical (S), Indo-Malayan (I), Palearctic (P), and Australasian (U). Outgroups were not included when inferring ancestral regions for the Ceratinini.

RESULTS

Phylogenetic analyses

The maximum parsimony bootstrap analysis (Fig. 1) showed very low levels of support for nearly all nodes except those that correspond to subgeneric groupings. The monophyly of the Ceratinini was well supported and all subgenera except *Ceratina sensu stricto* were resolved as monophyletic clades. The main features of the bootstrapped topology suggest *Neoceratina* as sister to all other subgenera and *Ceratina s. s.* basal to the remaining subgenera. The apical nodes of the tree suggest the Asian subgenera *Lioceratina* and *Ceratinidia* as well as American subgenera *Ceratinula, Calloceratina* and *Zadontomerus* are the most recently derived clades.

The BI consensus phylogram is shown in Figure 2. Posterior probability (PP) support is indicated for each node where support was less than 100%. Monophyly of the ceratinines was strongly supported (100 PP), and there was high support (94 PP) for *Neoceratina* as sister clade to the remaining subgenera in our sample. Conversely, there was weak support (54 PP) for the placement of *Megaceratina* at the base of the African clade and the placement of *Ceratina s. s.* is polyphyletic around *Copoceratina* with weak support (69 PP).

Hirashima, Ctenoceratina and *Simioceratina* formed a weakly supported clade (64 PP). The *Hirashima* clade presented two strongly supported (100 PP) African clades with a Malagasy clade contained within one of these. *Ctenoceratina* and *Simioceratina* were recovered as strongly supported sister groups (100 PP). The Malagasy *Malgatina azurea* and four Palaearctic species placed in *Euceratina* were recovered as a strongly supported (100 PP) monophyletic grouping. The position of an undescribed African species whose morphology justifies subgeneric ranking (and referred to here as 'New subgenus') with respect to *Pithitis* had moderate support (84 PP). Monophyly of the *Pithitis* group was well supported (100 PP), containing a strongly supported Asian (99 PP) and African (100 PP) clade. The node joining the Asian species contained in *Ceratinidia* and *Lioceratina,* and the American species in *Zadontomerus*, *Calloceratina* and *Ceratinula* was highly supported (100 PP). The placement of *Lioceratina* and *Ceratinidia* were highly supported (100 PP), however the relationship among the three American subgenera was ambiguous (48 PP). Within the *Neoceratina* clade the Mauritian and Malaysian specimens were identical across all three gene regions suggesting these are one species with a recent translocation to Mauritius (see Discussion below)

Subgeneric groups, with the exception of *Ceratina s. s.,* were all highly supported clades (100 PP). The low PP support values in our BI analysis generally coincided with very short basal branch lengths in the consensus phylogram (Fig. 2). Interestingly, these nodes

involve bifurcations among clades with very different global distributions (viz. Madagascar and Palearctic, Africa and Asia, Asia+North America). Understanding these bifurcation events in an historical biogeographical scenario requires that we have some indication of the likely ages of key nodes, and we explore this in the following section.

Molecular dating

We used penalized likelihood transformation of the Bayesian consensus phylogram to produce a chronogram (Fig. 3), which also indicates the geographic distribution of each species. Results from our BEAST relaxed clock analysis for key-node estimated ages and HPDs are given in Table 2 where they are directly compared to results from the r8s analysis. We found broad concordance in estimated ages from the two approaches suggesting that given the fossil calibration points available and the species sampled in this study age estimates are robust to the methods employed. Age estimates were largely identical with the exception of the root node of *Hirashima* and subsequent Malagasy bifurcations (Table 2). This suggests that age estimates are sensitive between methods for recent nodes. For the remainder of this section and the discussion we refer to r8s age estimate as these are most comparable in methodology to phylogenetic literature on other bee groups.

The penalised likelihood point estimate for the crown age of the tribe Ceratinini is 47±8.8 Mya and the relaxed clock analysis gave a very similar result (Table 2). The divergence of the New World *Ceratinula/Zadontomerus* lineage from the lineage leading to the Asian *Lioceratina/Ceratinidia* was estimated at about 32±8.1 Mya and the latter Asian clade had a crown age of 27±7.5 Mya. Relaxed clock dates for these nodes were very similar (Table 2). Dispersal from Africa into Madagascar occurred in at least two lineages. First, the lineage leading to the Malagasy subgenus *Malgatina* split from an African clade some 25±8.4 Mya. Second, the crown group age for the African/Malagasy *Hirashima* was 23±9.3 Mya.

Relaxed clock estimates for these two nodes were substantially younger, though confidence intervals were all overlapping (Table 2). It should be remembered that the above estimates are based on two calibration points that are likely to be conservative, so that actual dates may be older, but are unlikely to be younger. When we increased the set age of the root connecting the corbiculates to the Xylocopinae clades from 90 to 120 Mya, we found that the estimated ages of internal nodes increased proportionately and in a linear manner, as Chenoweth et al. (2007) found in their allodapine study. This is probably because the estimated ages for the internal minimum-age calibration points were much older than the set minimums, so that the fixed age of the root node had the strongest effect on scaling the tree.

Biogeographic analyses

Ancestral geographic ranges were estimated for eight well supported nodes in the Bayesian tree (Fig. 3). BayesMultiState analyses allowed for free rates of biogeographic exchange between the seven ecozones. Analyses suggest an Afrotropical origin at the root of the Ceratinini (node A) where the reconstructed probability for an Afrotropical origin was more than three times greater than for any alternative region. The centre of origin for *Neoceratina* (node B) is less clear, with the Australasian, Indo-Malayan and Palearctic regions having probabilities ranging from 16 – 33% for being ancestral regions. These three regions are geographically contiguous and several species in our analyses occurred in more than one region. Our analyses therefore do not permit us to infer in which ecozone the *Neoceratina* lineage arose, but support for an Afrotropical origin of Ceratinini suggests that *Neoceratina* arose from a north-eastern dispersal from Africa. The next-most distal bifurcations after the split of *Neoceratina* from the other ceratinine clade all have low PP support. This means that we are unable to be confident about related dispersal events among the associated regions. However, strong support for subgeneric nodes and patterns in their

regional distributions indicate an African origin with early dispersals extending into all other ecozones prior to 20 Mya.

Distribution ranges suggest three dispersal events subsequent to African diversification. First, the centre of origin of *Hirashima* (node D) suggests an African origin with two dispersal events into Madagascar or a Malagasy origin with two dispersals westward to Africa. Second, the analyses indicated that a Palearctic origin is more likely than a Malagasy origin for the *Malgatina* and *Euceratina* common ancestor (node F) though any dispersals between these regions would have required a presence in Africa with subsequent extinction in that region. Third, *Pithitis* was found as two distinct Afrotropical and Indo-Malayan clades and the root node of these clades had a higher likelihood of comprising an Indo-Malayan lineage than being Afrotropical (node G). Subsequent dispersal out of Africa into the Holarctic was supported by node H, suggesting an Afrotropical to Neotropical, or Indo-Malayan to Neotropical genesis of the New World subgenera and a Palearctic to Indo-Malayan expansion and genesis of *Lioceratina* and *Ceratinidia* (node I).

Diversification rates over time

The lineage through time (LTT) for the consensus chronogram (Fig. 4) showed a very similar pattern to that of the randomly chosen post-burnin trees with a strong deviation from the linearity that would otherwise be expected if speciation/extinction ratios had remained constant over time. The plots suggest higher rates of cladogenesis up until about 37 Mya, with a levelling off in rates after this time. The graph suggests a further slowing of cladogenesis from about 5 Mya, but this could reflect, at least partially, our taxon sampling regime where we largely avoided inclusion of taxa that were not clearly morphologically distinct. While the LTT plot for the consensus chronogram showed some potentially

interesting deviations from linearity between about 30 Mya and the present, variation in the post-burnin LTT plots makes it difficult to discern any clear patterns.

Although LTT plots provide a graphical means for representing diversification rates over time they do not permit any numerical interpretation in themselves. Our estimates of the gamma parameter do, however, allow this but with some strong limitations. The distribution of gamma values for 1000 randomly selected post-burnin trees is contrasted with gamma values based on 5000 randomly generated trees, assuming an actual clade size of 339 terminal taxa (Integrated Taxonomic Information System on-line database, http://www.itis.gov) and reduced to 71 sampled species, in Figure 5. It is not possible to statistically compare these two distributions since the empirically derived post-burnin trees do not represent independent samples from a population. Furthermore, the number of post-burnin trees and the number of simulated trees can be arbitrarily large, so that even very small differences in their central tendency could be made significant by simply increasing the post-burnin generations or the number of simulated trees. Given this caveat, the two distributions clearly differ in their central tendencies, with the empirically-derived values tending to lower values, which indicate declining rates of cladogenesis over time. This means that our gamma values suggest that diversification rates were higher in the past than would be expected by under-sampling of taxa alone. This concords with our LTT plots and branch lengths separating basal nodes for the consensus chronogram.

DISCUSSION

Phylogeny and evolution of the Ceratinini

The only molecular study of *Ceratina* phylogenetics to date (Cronin 2004) used a restricted number of species from the Indo-Malayan and Palearctic regions and did not explore divergence times. While Terzo's morphology-based study (2000) examined a large

proportion of the described subgenera, the morphological characters used did not permit resolution of many key relationships. Our study takes advantage of an unprecedented DNA sequence database of newly sequenced *Ceratina* species from both the Old and New Worlds. Our resulting phylogenetic hypotheses show some convergences with previous studies, but there are also sharp contrasts. These differences have some important consequences for our understanding of the evolutionary history of this group of bees.

Our analyses recovered all included subgenera as monophyletic groups with the exception of *Ceratina s. s.*, which was paraphyletic. Terzo and Rasmont (2007) have recently described a new subgenus *Dalyatina* with one Mediterranean and six sub-Saharan species from species groups within *Ceratina s. s.*; *C. aloes* and *C. subquadrata* are represented here and *Dalyatina* appears to be polyphyletic (Fig. 2). *Ceratina s. s.* is systematically problematic, found worldwide and contains many species groups (Yasumatsu and Hirashima 1969; Hirashima 1971; Pauly et al. 2001; Eardley and Daly 2007). This subgenus is a potentially important group for understanding the evolutionary patterns in the tribe, but the current taxonomy is clearly in need of revision.

In order to infer a New or Old World origin for this ubiquitous tribe it is important to understand the relationships between the New and Old World subgenera, and it is significant that our results are incongruent with the earlier morphology-based studies by Terzo (2000). We inferred that *Neoceratina* is sister group to all other included ceratinines, including the clade from which the Afrotropical subgenera *Megaceratina* and *Ceratina s. s.* evolved (Figs. 1-3). Conversely, Terzo (2000) recovered the New World subgenus *Zadontomerus* as sister clade to a Holarctic clade in which the wide-spread Old World subgenus *Neoceratina* and then the New World subgenus *Ceratinula* evolved. On the other hand, our molecular analyses and the previous morphology-based analyses (Terzo 2000) of the Ceratinini produced broadly similar topologies for the African clades. Both studies strongly support

Hirashima as sister to the *Ctenoceratina* + *Simioceratina* clade. Moreover, a close sister-subgenus relationship between *Malgatina* and *Euceratina* is supported by both approaches. Terzo's phylogeny was largely unresolved for older nodes, with a basal polytomy including numerous Old World subgenera, so that inferring origins and subsequent dispersal patterns was difficult. Our results indicated that the phylogenetic signal in our molecular data set was stronger for these deeper nodes, and provides strong support for an Old World origin with a single dispersal into the New World followed by radiation there and no back-dispersal to the Old World. The historical biogeography of the tribe will be discussed in more detail in the following section.

Age and origin of the Ceratinini

Incomplete sampling of subgenera in our study could create some problems for inferring ancestral regions if missing subgenera are geographically biased. We did not have specimens for seven of the 23 subgenera. These missing subgenera contain about 30 species from a total number of about 200 described species that Michener (2007) ascribes to each subgenus, or about 15% of described ceratinines. In terms of geographic representation our samples do not appear to be biased: we included three of the five New World subgenera, nine of the eleven subgenera with representatives in Africa and Madagascar, three of the five subgenera with representatives in the Indo-Malayan region (although two of the missing Indo-Malayan subgenera are monotypic), and three of the four subgenera with representatives in the Palaearctic.

Our results suggested an African origin for the tribe approximately 47 Mya. An African origin is similar to that proposed for the closely related and similarly aged (~ 47 Mya) bee tribe Allodapini (Schwarz et al. 2006). However, both the inferred origin times and regions of origin for these two tribes is complicated by a key factor, the fossil tribe

Boreallodapini. Three species from this tribe are recorded from Baltic amber dated at 44.1±1.1 Mya (Engel 2001) and Engel (2001) proposed that the Boreallodapini forms the sister tribe to the Allodapini, with the Ceratinini being the next-most basal tribe in the Xylocopinae. An Oriental origin was proposed for the closely related and similarly aged (~ 45 Mya), and globally distributed large carpenter bee genus *Xylocopa* (Leys et al. 2002).

Our results preclude a New World origin for the Ceratinini since the Nearctic and Neotropical clades are clearly distal in our phylogeny. A Eurasian origin would be concordant with the existence of the Baltic fossil tribe Boreallodapini and a Palearctic/Indo-Malayan origin for *Neoceratina*. However, an African origin for the tribe seems more likely since a Eurasian origin would require minimal diversification of what would be a relictual Eurasian *Neoceratina* clade, with a single dispersal into Africa, followed by large scale diversification there and subsequent dispersals out of Africa. Moreover, both biodiversity considerations (Michener 1969) and morphological phylogenetics (Terzo 2000) of the ceratinines have suggested an African origin with subsequent dispersals into Asia and the New World. Given an African origin of the Ceratinini, our analyses suggest multiple dispersals out of Africa, represented by the *Neoceratina* clade, the clade leading to *Ceratina minutula*, the clade leading to *Euceratina*, and the clade leading to the Asian *Ceratinidia/Lioceratina* and the New World subgenera. Presently, we cannot be certain of the number and direction of these dispersal events due to the low support for basal nodes.

The New World ceratinines present two possible biogeographic scenarios. The sister relationship between the New World subgenera and the Old World Asian *Ceratinidia* and *Lioceratina* support the notion of a Bering Strait dispersal some 32 Mya. This dispersal timing is similar to that of two other cosmopolitan bee genera *Bombus* and *Xylocopa*, both of which are inferred to have had the same dispersal route across the Bering Strait, approximately 20 and 34 Mya respectively (Hines 2008; Leys et al. 2002). Conversely, the

low support at basal nodes and African antecedents cannot preclude an Afrotropical to Neotropical dispersal as found in some halictid bees (Danforth et al. 2008). Southern hemisphere long range oceanic dispersals have also been proposed for stem nesting allodapine bees (Schwarz et al. 2006).

The Ceratinini are of cosmopolitan distribution whereas their sister tribe, the Allodapini, are found only across the Old World and with limited representation in the Palearctic. In contrast to the Ceratinini, *Xylocopa* (Leys et al. 2002) and *Bombus* (Hines 2008), the Allodapini (Schwarz et al. 2006) and Ctenoplectrini (Schaefer and Renner 2008) are limited to an Old World distribution. This limited distribution could be explained if dispersal in Laurasia was limited by requirements for tropical or subtropical habitats, and indeed Eurasian Allodapini and Ctenoplectrini are found in low latitude landscapes. The only Eurasian allodapines that occur outside tropical and subtropical areas are in the rare Middle Eastern genus *Exoneuridia*. The only *Exoneuridia* species where nests have been found is *E. hakkariensis* and it is unique among allodapines by nesting in rock cavities on cliff faces (Schwarz unpub. data). Conversely the Ceratinini, *Xylocopa* and *Bombus* are found across the Holarctic with species distributions into the boreal forests above 50°N latitude (Bishop and Armbruster 1999; Janzon and Svensson 1988; Malyshev 1931). These species are known for their cold hardiness and resilience (Sakagami et al. 1981; Somanathan and Borges 2001; Corlett 2001, 2004) a requisite adaptation to surviving northern climates. In addition, the Ceratinini and *Xylocopa* have truly cosmopolitan ranges with more flexible habitat preferences, also being able to spread in warm habitats (Michener 1979). Conversely, *Bombus* do not extend into tropical areas and therefore has a less cosmopolitan range than Ceratinini. The remarkable range covering both boreal and tropical habitats and physiological adaptation to a mix of cold and thermo-tolerance make the Ceratinini and *Xylocopa* of interest for further studies on diversification and dispersal abilities of the bees.

Malagasy bee fauna

There have been at least two dispersals of *Ceratina* from Africa to Madagascar. The first dispersal of ceratinines across the Mozambique Channel is estimated at 25 Mya giving rise to the endemic *Malgatina*. This was followed by a second and perhaps third dispersal and radiation by *Hirashima* 23 and 9 Mya. Our analyses indicate that a Malagasy origin and subsequent dispersal westward into Africa, or two distinct dispersals from Africa to Madagascar, are equally parsimonious.

The endemism of the Malagasy fauna has been well documented in recent years (Pauly et al. 2001). Phylogenetic studies have shown recent and recurrent dispersal of African fauna into Madagascar across the 450km wide Mozambique Channel. Madagascar reached its current distance from Africa some 80 Mya, yet some fauna appear to have arrived more recently (Yoder and Nowak 2006). Rafting and wind dispersion are common hypotheses for this long-range oceanic dispersal.

The bee fauna of Madagascar has recently been surveyed, with Pauly and colleagues (2001) documenting nine endemic genera, and Chenoweth and colleagues (2008) describing an additional endemic genus. Molecular dating analyses indicate that all of the inferred African-Malagasy bee dispersal events were less than 30 Mya. Furthermore, there are no bee tribes in Madagascar that are not present in Africa (Pauly et al. 2001), suggesting that the distinctive nature of the Malagasy bee fauna is unlikely to have a very ancient origin (Eardley et al. 2009). The recent and recurrent origins of Malagasy bee genera may instead reflect moderately old to recent events followed by radiation in a new environment. The multiple dispersals of Ceratinini from Africa to Madagascar is similar to *Charaxes* butterflies, where there have been at least three dispersal events over the period of 20-13 Mya (Aduse-Poku et al. 2009).

One major puzzle that arises from our analyses is the monophyly of the Palearctic *Euceratina* and Malagasy *Malgatina* species without any African representation of either subgenus. Comparison of 51 morphological characters across the Ceratinini suggested that *Euceratina* and *Malgatina* are sister subgenera nested within the African taxa (Terzo 2000). The elaborate male genitalia, metallic colouration and dense punctuation are but a few of the commonalities. It is possible that the *Malgatina* in Madagascar are truly indigenous and evidence of dispersal from Eurasia to Madagascar has been lost through extinction in Africa or that dispersal did not involve an African route. It is difficult to see how the lineage leading to *Malgatina* could have reached Madagascar without an African presence, suggesting that such an African clade must have become extinct. This possibility was also suggested by Terzo (2000) in his analysis of *Euceratina* exemplars and the Malagasy *Malgatina azurea*. Conversely, anthropogenic dispersal seems likely to explain the occurrence of *Neoceratina dentipes* in Mauritius. *Neoceratina dentipes* is abundant and wide spread across Asia but unknown in Africa. Finding the same species off the coast of Africa, therefore, suggests anthropogenic dispersal from Malaysia to Mauritius, a known trade route over the past century or more (Mountain and Proust 2000; Rudwick 2005).

Rapid radiations

Ancient rapid radiations, defined as rapid speciation over short evolutionary time scales, have been found in numerous plant and animal groups (Whitfield and Lockhart 2007). The phylogenetic topology is one of compressed cladogenesis compared to that expected by constant diversification (Rokas et al. 2005). Rapid radiations are especially recurrent across insect orders and many of these seem to correspond with angiosperm radiations of the Cretaceous and Tertiary including Lepidoptera and their parasitoids, phytophagous Coleoptera, and corbiculate bees (reviewed in Whitfield and Kjer 2008). Phylogenies of

ancient groups often lack resolution during times of rapid radiation generating patterns of molecular and morphological changes that are difficult to resolve phylogenetically. Here we observed signature short basal branch lengths (Fig. 3) and rapid cladogenesis (Figs. 4 and 5) suggesting high rates of diversification during early evolution of the ceratinines.

Comparing phylogenies among closely related groups can reveal the differences in rates of cladogenesis and signs of relaxed constraint in some taxa. The poor resolution of the basal nodes of the Ceratinini using the same molecules as its relatively well resolved sister tribe Allodapini, suggests that the ceratinines are somewhat unique; radiating rapidly and potentially relaxed from evolutionary constraints seen in the Allodapini. Thus, the aforementioned taxonomic uncertainty among early African ceratinines is not so surprising considering the marked morphological variation among subgenera; species ranging from 2.2 to 12.5 mm in body length, with an array of: dull black to metallic blue green colouration, smooth to punctuate surface sculpturing, hairless to plumose appendages, and elaborate abdominal setae, tegument maculation, and clypeal protrusion unique among subgeneric groups. Conversely, allodapine bees are relatively monomorphic possessing some size and morphological variation, but to a much lesser extent than the ceratinines. Revision of poorly resolved microgastrine wasps found that additional genes did not and, after modelling putative genes, likely will never resolve short internal branches (Banks and Whitfield 2006). However, these authors do suggest combining molecular and morphological characters to increase support for deep branches in the phylogeny. This approach is certainly worth pursuing for the ceratinines following further taxonomic revision of the group.

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Table 1: List of species sequenced for this study along with Genbank accession numbers and their collection location. Species distributions are indicated in any ecozone as: A = Afrotropical, I = Indo-Malayan, N = Nearctic, M = Madagascar, P = Palearctic, S = Neotropical, and U = Australasian. Outgroups (*Manuelia* spp.) were not used for biogeographic analyses thus distributions are omitted with dashes.

Subgenus	Species	Distribution	Collection location	Accession Numbers		
				EF1α-F2	Cyt *b*	COI
Calloceratina	Panama sp	S	Panama	GU321643	GU321574	GU321508
Calloceratina	blue sp	S	Argentina	GU321639	N/A	GU321504
Ceratina	*minutula*	I	Turkey	GU321671	GU321601	GU321536
Ceratina	*subquadrata*	A	South Africa	GU321669	GU321599	GU321534
Ceratina	*braunsi*	A	South Africa	N/A	GU321597	GU321532
Ceratina	*rhodura*	A	South Africa	GU321672	GU321602	GU321537
Ceratina	*aloes*	A	South Africa	GU321670	GU321600	GU321535
Ceratina	*perpolita*	A	South Africa	GU321673	N/A	GU321538
Ceratina	*speculifrons*	A	Kenya	GU321668	GU321598	GU321533
Ceratinidia	*papuana*	I U	Malaysia	GU321609	GU321546	GU321474
Ceratinidia	*bowringi*	I	India	GU321611	GU321548	GU321476
Ceratinidia	*hieroglyphica*	I	India	GU321614	GU321551	GU321479
Ceratinidia	*moderata*	I	India	GU321607	GU321544	GU321472
Ceratinidia	*bryanti*	I	Malaysia	GU321612	GU321549	GU321477
Ceratinidia	*japonica*	P	Japan	GU321605	GU321542	GU321470
Ceratinidia	*okinawana*	I P	Japan	GU321613	GU321550	GU321478
Ceratinidia	*nigrolateralis*	I	Malaysia	GU321606	GU321543	GU321471
Ceratinidia	*accusator*	I	Malaysia	GU321610	GU321547	GU321475
Ceratinidia	*cognata*	I	Malaysia	GU321608	GU321545	GU321473
Ceratinula	*breviceps*	S	Bolivia	GU321642	GU321573	GU321507
Ceratinula	Paraguay sp	S	Paraguay	GU321635	GU321568	GU321500
Ceratinula	*cockerelli*	N	U.S.A.	GU321641	N/A	GU321506
Copoceratina	*minuta*	A	South Africa	GU321667	N/A	GU321531
Ctenoceratina	*pencillata*	A	Kenya	GU321632	GU321565	GU321497
Ctenoceratina	*penicilligera*	A	Kenya	GU321629	N/A	GU321494

Ctenoceratina	*malindae*	A	Kenya	GU321631	GU321564	GU321496
Ctenoceratina	*ericia*	A	Zambia	GU321624	GU321559	GU321489
Ctenoceratina	*lineola*	A	Tanzania	GU321630	GU321563	GU321495
Ctenoceratina	*bilobata*	A	Kenya	GU321626	GU321561	GU321491
Ctenoceratina	Zambia sp	A	Zambia	GU321625	GU321560	GU321490
Ctenoceratina	*rufigastra*	A	Kenya	GU321628	N/A	GU321493
Ctenoceratina	Kenya sp	A	Kenya	GU321627	GU321562	GU321492
Euceratina	*chrysomalla*	P	Turkey	GU321620	N/A	GU321485
Euceratina	*mandibularis*	P	Turkey	GU321617	GU321554	GU321482
Euceratina	*tibialis*	P	Turkey	GU321619	GU321556	GU321484
Hirashima	S Africa sp1	A	South Africa	GU321618	GU321555	GU321483
Hirashima	S Africa sp2	A	South Africa	GU321646	GU321576	GU321511
Hirashima	Malagasy sp1	M	Madagascar	GU321644	N/A	GU321509
Hirashima	Malagasy sp2	M	Madagascar	GU321645	GU321575	GU321510
Hirashima	*lativentris*	M	Madagascar	GU321649	GU321579	GU321514
Hirashima	Zambia sp1	A	Zambia	GU321650	GU321580	GU321515
Hirashima	Zambia sp2	A	Zambia	GU321647	GU321577	GU321512
Lioceratina	*flavolateralis*	I	Malaysia	GU321648	GU321578	GU321513
Malgatina	*azurea*	M	Madagascar	GU321615	GU321552	GU321480
Neoceratina	*australensis*	U	Australia	GU321616	GU321553	GU321481
Neoceratina	*dentipes*	I P U	Mauritius	GU321633	GU321566	GU321498
Neoceratina	*dentipes*	I P U	Malaysia	GU321651	GU321581	GU321516
Neoceratina	*propinqua*	I	India	GU321655	GU321585	GU321520
Neoceratina	Solomons_sp	U	Solomon Islands	GU321652	GU321582	GU321517
Neoceratina	*bispinosa*	P	Israel	GU321657	GU321587	GU321521
Neoceratina	*satoi*	P	Japan	GU321653	GU321583	GU321518
New subgenus	sp	A	Kenya	GU321656	GU321586	N/A
Pithitis	*unimaculata*	I	Malaysia	GU321654	GU321584	GU321519
Pithitis	*fastigata*	A	Zambia	GU321674	GU321603	GU321539
Pithitis	*waini*	A	Zambia	GU321665	GU321595	GU321529
Pithitis	*citriphila*	A	Zambia	GU321661	GU321591	GU321525
Pithitis	*smaragdula*	I P	Indonesia	GU321659	GU321589	GU321523

Pithitis	*tarsata*	A	Zambia	GU321666	GU321596	GU321530
Pithitis	*nasalis*	A	Swaziland	GU321664	GU321594	GU321528
Pithitis	*binghami*	I	India	GU321662	GU321592	GU321526
Pithitis	*Kenya sp*	A	Kenya	GU321663	GU321593	GU321527
Simioceratina	*lunata*	A	Zambia	GU321658	GU321588	GU321522
Simioceratina	*tanganyicensis*	A	Tanzania	GU321660	GU321590	GU321524
Simioceratina	*moerenhouti*	A	Kenya	GU321621	GU321557	GU321486
Zadontomerus	*dupla*	N	U.S.A.	GU321623	N/A	GU321488
Zadontomerus	*floridana*	N	U.S.A.	GU321622	GU321558	GU321487
Zadontomerus	*calcarata*	N	Canada	GU321634	GU321567	GU321499
Zadontomerus	*strenua*	N	Canada	GU321640	GU321572	GU321505
Zadontomerus	*cyaniventris*	S	Cuba	GU321638	GU321571	GU321503
Manuelia	*gayi*	-	Chile	GU321636	GU321569	GU321501
Manuelia	*gayatina*	-	Chile	GU321637	GU321570	GU321502

Table 2: Comparison of crown-age estimates for some key clades, using penalized likelihood (r8s) and relaxed clock (BEAST) methods.

	Penalized likelihood (r8s)		Bayesian relaxed clock (BEAST)	
	mean	95% CI	mean	95% CI
Ceratinini	47	39-56	47	32-63
Hirashima	23	14-32	15	6-24
Hirashima lativentris + Malagasy sp 1	9	5-13	4	0-9
Simioceratina + *Ctenoceratina*	32	23-40	24	14-36
Malgatina + *Euceratina*	25	17-33	19	4-36
Euceratina	15	8-22	12	2-27
Pithitis	19	12-25	26	8-43
New World subgenera + *Lioceratina* + *Ceratinidia*	32	26-40	32	19-47
Lioceratina + *Ceratinidia*	23	16-30	25	15-38

FIGURE CAPTIONS

Figure 1. MP bootstrap tree. Bootstrap support is indicated for each node except nodes with 100% support.

Figure 2. Consensus phylogram from Bayesian analysis. Posterior probabilities are indicated for each node.

Figure 3. Chronogram of the Ceratinini derived from penalized likelihood transformation of the consensus Bayesian phylogram. Geographic distributions of each species are colour coded according to the map. BayesMultistate analysis of ancestral geographic reconstructions indicated as pie charts indicating the relative likelihoods of each region at respective nodes (A-I).

Figure 4. Lineage through time plot of Ceratinini cladogenesis over time. Grey lines represent 49 randomly selected post-burnin samples and the blue line represents the LTT plot from the consensus chronogram.

Figure 5. Gamma distributions of sampled (71 species) versus simulated (339 species) phylogenies. Top: Distribution of 1000 randomly-sampled post-burnin trees of the 71 ceratinine species sampled in this study. Bottom: Gamma distribution of 5000 trees based on described ceratinine diversity (339 species) with all but 71 terminals randomly deleted. Lower gamma values indicate increasing rates of cladogenesis closer to the root node.

Figure 1:

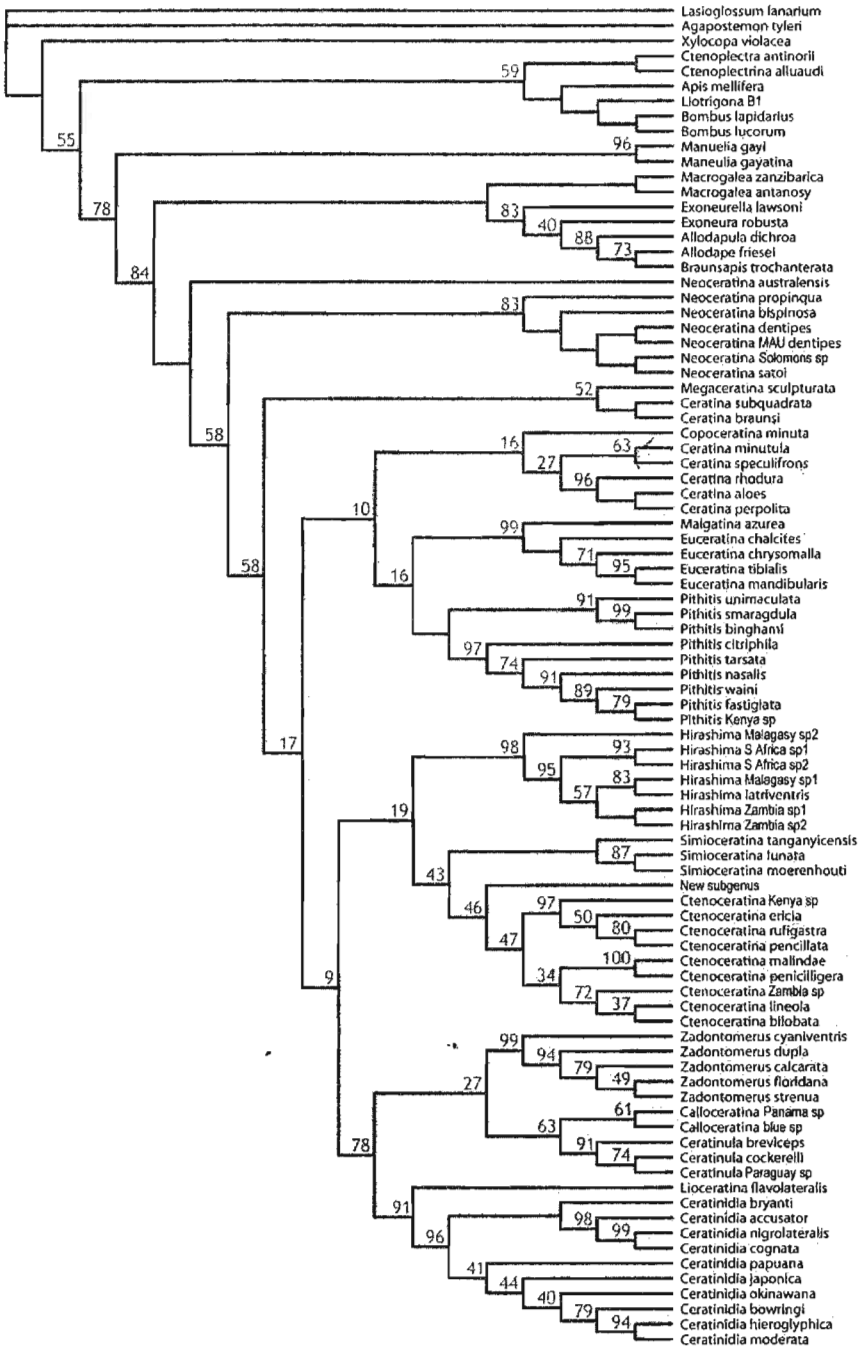
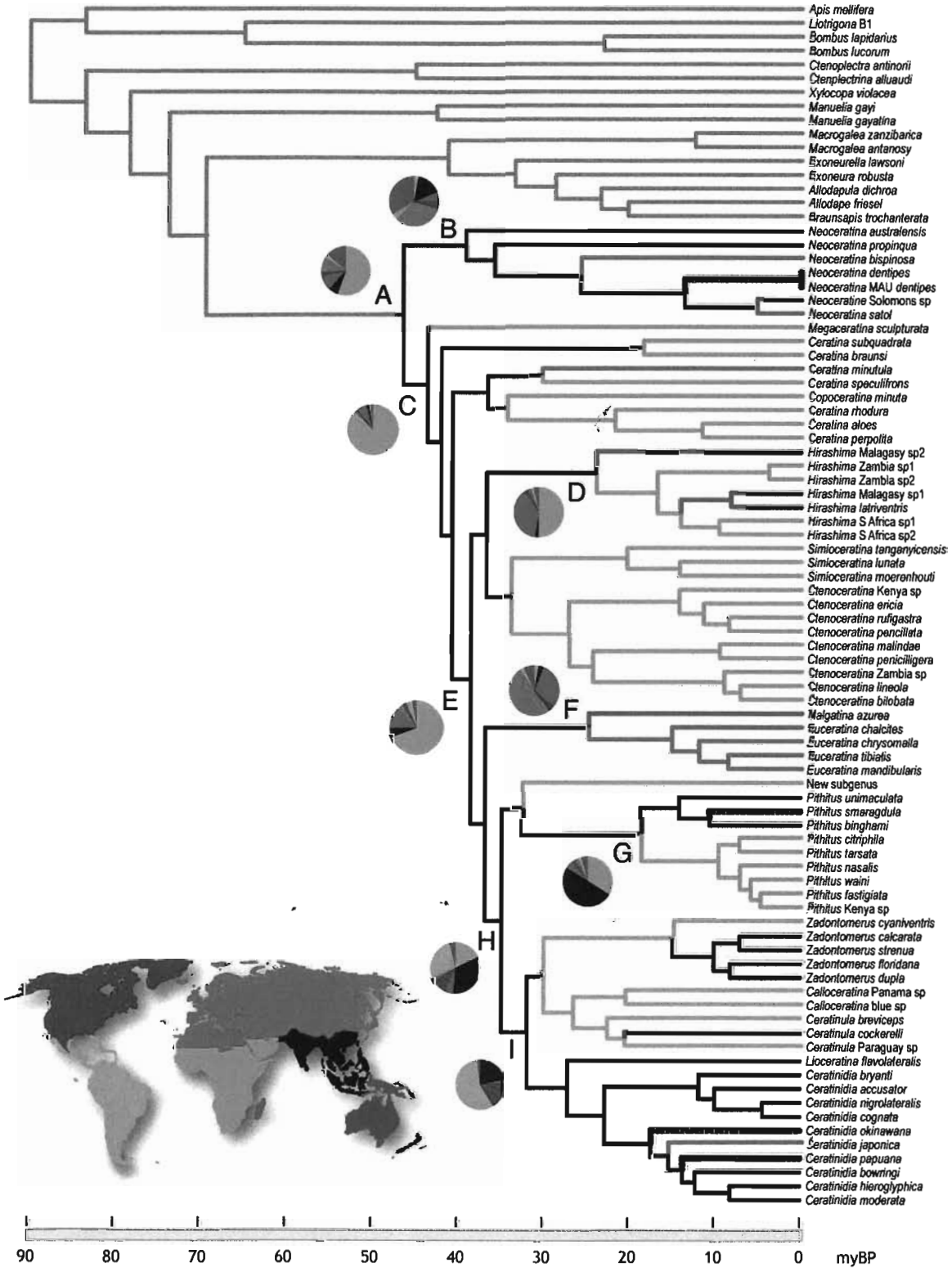

Lasioglossum fanarium
Agapostemon tyleri
Xylocopa violacea
Ctenoplectra antinorii
Ctenoplectrina alluaudi
Apis mellifera
Liotrigona B1
Bombus lapidarius
Bombus lucorum
Manuelia gayi
Maneulia gayatina
Macrogalea zanzibarica
Macrogalea antanosy
Exoneurella lawsoni
Exoneura robusta
Allodapula dichroa
Allodape friesei
Braunsapis trochanterata
Neoceratina australensis
Neoceratina propinqua
Neoceratina bispinosa
Neoceratina dentipes
Neoceratina MAU dentipes
Neoceratina Solomons sp
Neoceratina satoi
Megaceratina sculpturata
Ceratina subquadrata
Ceratina braunsi
Copoceratina minuta
Ceratina minutula
Ceratina speculifrons
Ceratina rhodura
Ceratina aloes
Ceratina perpolita
Malgatina azurea
Euceratina chalcites
Euceratina chrysomalla
Euceratina tibialis
Euceratina mandibularis
Pithitis unimaculata
Pithitis smaragdula
Pithitis binghami
Pithitis citriphila
Pithitis tarsata
Pithitis nasalis
Pithitis waini
Pithitis fastigiata
Pithitis Kenya sp
Hirashima Malagasy sp2
Hirashima S Africa sp1
Hirashima S Africa sp2
Hirashima Malagasy sp1
Hirashima latriventris
Hirashima Zambia sp1
Hirashima Zambia sp2
Simioceratina tanganyicensis
Simioceratina lunata
Simioceratina moerenhouti
New subgenus
Ctenoceratina Kenya sp
Ctenoceratina ericia
Ctenoceratina rufigastra
Ctenoceratina pencillata
Ctenoceratina malindae
Ctenoceratina penicilligera
Ctenoceratina Zambia sp
Ctenoceratina lineola
Ctenoceratina bilobata
Zadontomerus cyaniventris
Zadontomerus dupla
Zadontomerus calcarata
Zadontomerus floridana
Zadontomerus strenua
Calloceratina Panama sp
Calloceratina blue sp
Ceratinula breviceps
Ceratinula cockerelli
Ceratinula Paraguay sp
Lioceratina flavolateralis
Ceratinidia bryanti
Ceratinidia accusator
Ceratinidia nigrolateralis
Ceratinidia cognata
Ceratinidia papuana
Ceratinidia japonica
Ceratinidia okinawana
Ceratinidia bowringi
Ceratinidia hieroglyphica
Ceratinidia moderata

Figure 2:


Lassioglossum lanarium
Agapostemon tyleri
Apis mellifera
Liotrigona B1
Bombus lapidarius
Bombus lucorum
Ctenoplectra antinorii
Ctenoplectrina alluaudi
Xylocopa violacea
Manuelia gayi
Manuelia gayatina
Macrogalea zanzibarica
Macrogalea antanosy
Exoneurella lawsoni
Exoneura robusta
Allodapula dichroa
Allodape friesei
Braunsapis trochanterata
Neoceratina australensis
Neoceratina propinqua
Neoceratina bispinosa
Neoceratina dentipes
Neoceratina MAU dentipes
Neoceratina Solomons sp
Neoceratina satoi
Megaceratina sculpturata
Ceratina subquadrata
Ceratina braunsi
Ceratina minutula
Ceratina speculifrons
Copoceratina minuta
Ceratina rhodura
Ceratina aloes
Ceratina perpolita
Hirashima Malagasy sp2
Hirashima Zambia sp1
Hirashima Zambia sp2
Hirashima Malagasy sp1
Hirashima latriventris
Hirashima S Africa sp1
Hirashima S Africa sp2
Simioceratina tanganyicensis
Simioceratina lunata
Simioceratina moerenhouti
Ctenoceratina Kenya sp
Ctenoceratina ericia
Ctenoceratina rufigastra
Ctenoceratina penicillata
Ctenoceratina malindae
Ctenoceratina penicilligera
Ctenoceratina Zambia sp
Ctenoceratina lineola
Ctenoceratina bilobata
Malgatina azurea
Euceratina chalcites
Euceratina chrysomalla
Euceratina tibialis
Euceratina mandibularis
New subgenus
Pithitus unimaculata
Pithitus smaragdula
Pithitus binghami
Pithitus citriphila
Pithitus tarsata
Pithitus nasalis
Pithitus waini
Pithitus fastigiata
Pithitus Kenya sp
Zadontomerus cyaniventris
Zadontomerus calcarata
Zadontomerus strenua
Zadontomerus floridana
Zadontomerus dupla
Calloceratina Panama sp
Calloceratina blue sp
Ceratinula breviceps
Ceratinula cockerelli
Ceratinula Paraguay sp
Lioceratini flavolateralis
Ceratinidia bryanti
Ceratinidia accusator
Ceratinidia nigrolateralis
Ceratinidia cognata
Ceratinidia okinawana
Ceratinidia japonica
Ceratinidia papuana
Ceratinidia bowringi
Ceratinidia hieroglyphica
Ceratinidia moderata

Figure 3: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Bombus lapidarius
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Ctenplectrina alluaudi
Xylocopa violacea
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Manuelia gayatina
Macrogalea zanzibarica
Macrogalea antanosy
Exoneurella lawsoni
Exoneura robusta
Allodapula dichroa
Allodape friesei
Braunsapis trochanterata
Neoceratina australensis
Neoceratina propinqua
Neoceratina bispinosa
Neoceratina dentipes
Neoceratina MAU dentipes
Neoceratine Solomons sp
Neoceratina satoi
Megaceratina sculpturata
Ceratina subquadrata
Ceratina braunsi
Ceratina minutula
Ceratina speculifrons
Copoceratina minuta
Ceratina rhodura
Ceratina aloes
Ceratina perpolita
Hirashima Malagasy sp2
Hirashima Zambia sp1
Hirashima Zambia sp2
Hirashima Malagasy sp1
Hirashima latriventris
Hirashima S Africa sp1
Hirashima S Africa sp2
Simioceratina tanganyicensis
Simioceratina lunata
Simioceratina moerenhouti
Ctenoceratina Kenya sp
Ctenoceratina ericia
Ctenoceratina rufigastra
Ctenoceratina penicillata
Ctenoceratina malindae
Ctenoceratina penicilligera
Ctenoceratina Zambia sp
Ctenoceratina lineola
Ctenoceratina bilobata
Malgatina azurea
Euceratina chalcites
Euceratina chrysomalla
Euceratina tibialis
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Pithitus smaragdula
Pithitus binghami
Pithitus citriphila
Pithitus tarsata
Pithitus nasalis
Pithitus waini
Pithitus fastigiata
Pithitus Kenya sp
Zadontomerus cyaniventris
Zadontomerus calcarata
Zadontomerus strenua
Zadontomerus floridana
Zadontomerus dupla
Calloceratina Panama sp
Calloceratina blue sp
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Ceratinula cockerelli
Ceratinula Paraguay sp
Lioceratina flavolateralis
Ceratinidia bryanti
Ceratinidia accusator
Ceratinidia nigrolateralis
Ceratinidia cognata
Ceratinidia okinawana
Ceratinidia japonica
Ceratinidia papuana
Ceratinidia bowringi
Ceratinidia hieroglyphica
Ceratinidia moderata
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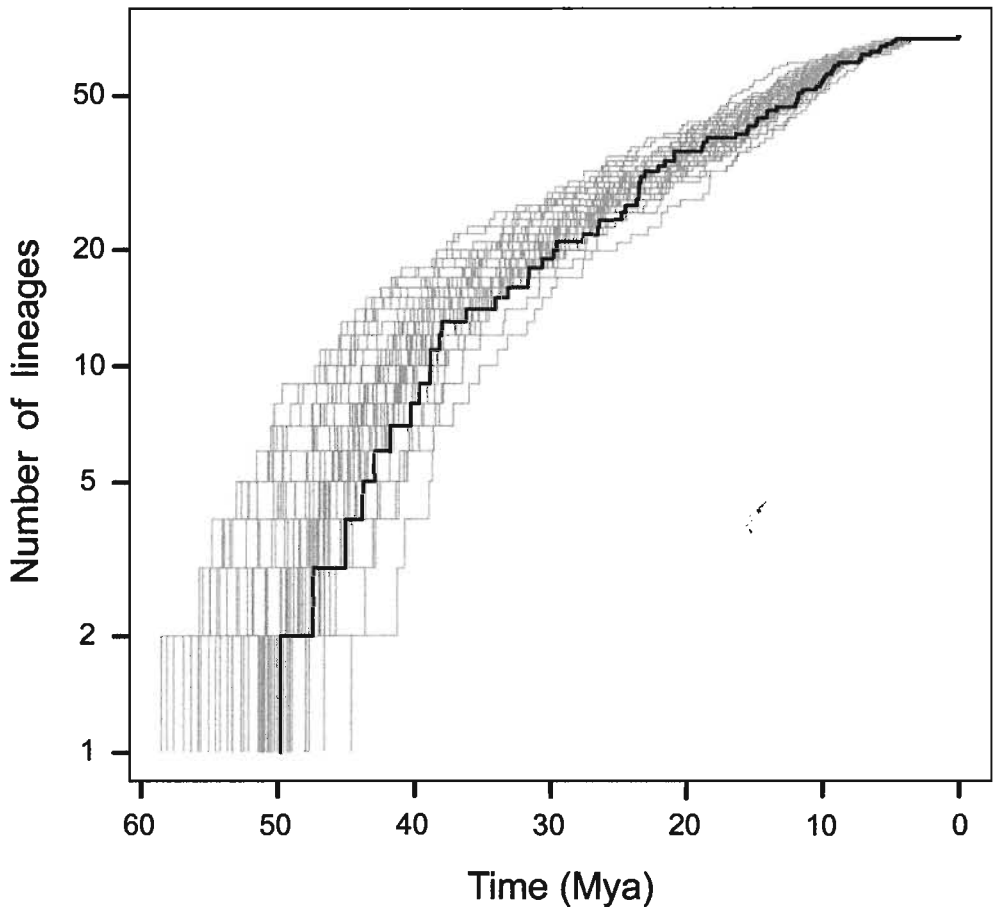
Figure 4:



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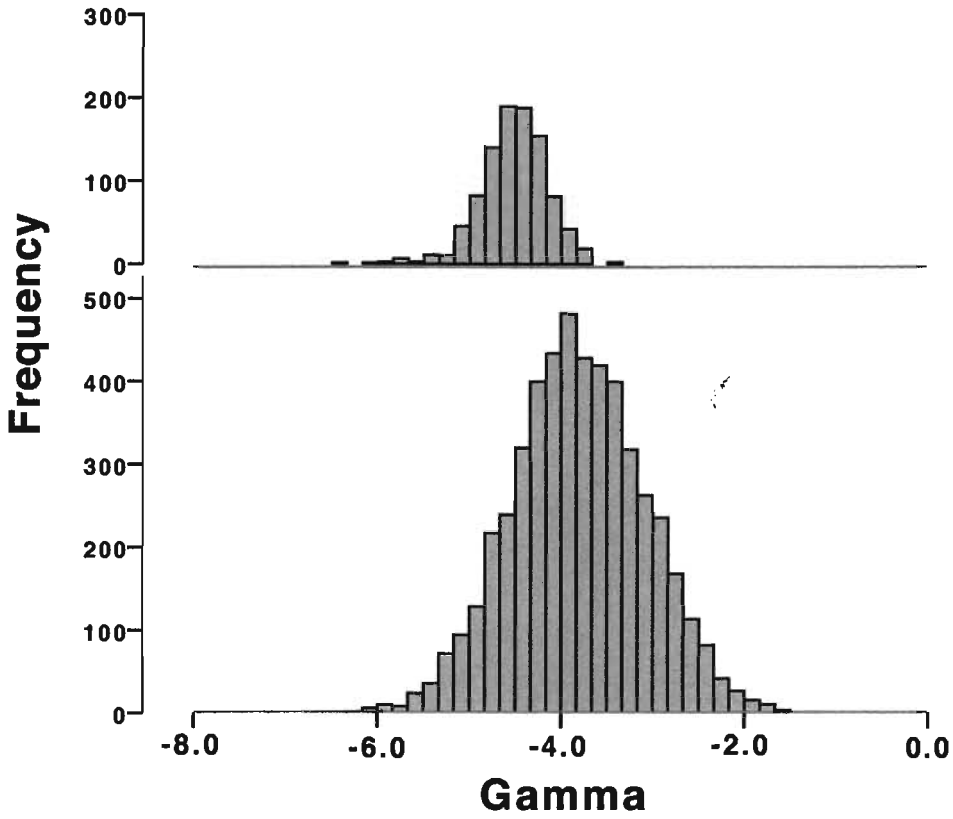
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Chapter 7: General Discussion

Subsociality is arguably the simplest form of social behaviour attained by animals (Tallamy and Wood 1986; Costa 2006). Subsociality is defined as prolonged parental care and parent-offspring interaction. Two preadaptations allow subsociality to arise: first nest loyalty, as it is difficult to defend or care for offspring dispersed through time and space; and second parental longevity, as parents need to survive long enough to potentially interact with their offspring throughout and/or after development (Tallamy and Wood 1986). Parental behaviour is recurrent throughout the animal kingdom yet rarely leads to higher social evolution as realized by the social Hymenoptera, the ants, bees and wasps. Other organisms attaining eusociality include the termites (Wilson 1971), aphids (Stern and Foster 1997), gall forming thrips (Crespi 1992), ambrosia beetles (Kent and Simpson 1992), snapping shrimp (Duffy 1996), flatworms (Hechinger et al. 2010), and naked mole rats (Jarvis 1981). These eusocial taxa are characterized by behavioural and reproductive differentiation, living together as adults, and performing some cooperation or task allocation among individuals (Michener 1974). Since subsocial taxa are quite common yet eusocial taxa are relatively scarce the question persists, what rare conditions act to facilitate the evolution of eusociality? Conversely, what prevailing selective forces retain the remaining taxa in a subsocial state?

Understanding the transition from subsociality to eusociality requires a group of closely related taxa possessing diverse sociobiology, ecology and biogeography. With numerous subsocial and social contrasts we can begin to understand the genetic underpinnings facilitating interaction and cooperative behaviour and the environmental factors creating staying incentives and strengthening group cohesion. Given that sociality has arisen most frequently and with greatest complexity within the Hymenoptera, they are key organisms to provide the most evolutionary contrasts. However, the social ants and

corbiculate bees evolved sociality >65 million years ago and their obligate sociality with morphological castes makes them less informative to understand the origin of social behaviour. Wilson and Hollodobler (2005) argue a 'point of no return' in social evolution when morphological and reproductive castes constrain social plasticity as seen in the absence of any social reversion in the highly social ants and termites (Wilson and Hollodobler 2005). To look back on highly social groupings and make inferences about their ancestral origins can be impossible as environmental and ecological factors that drove evolutionary processes in the past are not necessarily those of today.

Presocial taxa are fundamental to understanding the origins of sociality and can give insights into the evolutionary steps from solitary to social life. Key taxa with social plasticity persist within the halictine and xylocopine bees (Michener 1974; Wcislo 1997; Schwarz et al. 2007). Recent phylogenetic work on the halictids revealed three origins of eusociality and numerous reversions from social behaviour to solitary life within this family (Packer 1991; Wcislo and Danforth 1997; Danforth 2002). The Xylocopinae are a monophyletic grouping of xylophilous bees consisting of four tribes: the eldest Xylocopini, the intermediary Manueliini, and the youngest Allodapini and Ceratinini (Cardinal et al. 2010). Previous studies on the Xylocopini have shown that species display parasociality but never exhibit eusociality (Michener 1990). Limited work on the Manueliini suggests they are a relictual lineage (Daly et al. 1987) with only three extant solitary species (Flores Prado et al. 2008). Recent phylogenetic work on the allodapine bees has shown basal sociality with no reversion to solitary life (Schwarz et al. 2010; Chenoweth et al. 2007). Work on the Ceratinini is limited but species in this tribe exhibit the spectrum from solitary behaviour to eusociality within a narrow range of taxa (Michener 1985).

Here I propose the small carpenter bees, genus *Ceratina,* as model organisms to examine the origins of sociality. *Ceratina* are speciose, of cosmopolitan distribution, and

provide numerous contrasts with their diverse sociobiology, ecology and biogeography. *Ceratina* are key taxa to understanding the transition from subsocial to social behaviour, as all documented groups are long lived, nest loyal and tend to their young through development and some even after eclosion into adult stages (Sakagami and Maeta 1977). With the key subsocial preadaptations set, a few taxa have achieved sociality under the right combination of ecological and behavioural conditions.

Ecological Factors Contributing to Sociality

In Chapters 3 and 5 I examined three ecological factors proposed to contribute to the formation of social colonies in insects including nest limitation, natural enemies and climate (Lin & Michener 1972). First, since *Ceratina* have a very specific nesting substrate requirements, occupying dead, broken, pithy twigs, nesting resource limitation could be a driving factor and staying incentive for offspring to stay at the natal nest. If the probability of finding suitable nesting substrate elsewhere is limited offspring may remain nest loyal during the pre-hibernation phase or even remain at the nest during the nest initiation phase. Nest reuse is associated with social nesting in many species (Table 1).

Second, parasite and predator pressure can facilitate group nesting if a solitary foundress's brood is infected or consumed while she is off foraging. If retaining guards at the nest markedly reduces offspring mortality, staying at the natal nest could be favoured by selection. Social colonies are thought to be advantageous due to the benefits of lowering predator and parasite pressure (Lin and Michener 1972; Evans 1977; Andersson 1984). *Ceratina flavipes* and *C. japonica* nests exhibit increased brood cell mortality when orphaned and lower brood mortality when guarded, revealing that the presence of a mother at the nest entrance was effective in preventing mortality from small wasp and fly parasites, which were only present in orphaned nests (Sakagami and Maeta 1977). Evidence for the selective

benefit of group living is reported for *C. australensis* in which solitary nests can be extirpated by parasites but social colonies were never observed to succumb to total nest failure (Chapter 2; Rehan et al. 2010).

Third, across their cosmopolitan distribution *Ceratina* species and subgenera experience different geographic and climatic regions (Chapter 6; Table 1). In temperate regions of North America, the Palearctic and northern Asia, ceratinines have univoltine colony cycles allowing for mother-daughter interaction but no second brood in which a worker caste takes over foraging activities while the mother resumes reproduction. In tropical regions including but not limited to south-east Asia, northern Africa, the Iberian Peninsula, and Central and South America, extended active seasons facilitate bi- and multivoltine colony cycles and multigenerational overlap (Chapter 5; reviewed in Sakagami and Laroca 1971; Rehan et al. 2009). With maternal longevity and multiple consecutive broods mothers, daughters and siblings all have a chance to interact and influence each other's dispersal and reproduction decisions.

Ecological Factors Inhibiting Sociality

Some environmental conditions are known to facilitate solitary nest initiation. First, if nest resources are abundant, then females have ample opportunity to disperse and found solitary nests (Chapter 2; Rehan and Richards 2010). Likewise, environments with low parasite and predator load provide relaxed selection for social groups as solitary bee nests are seldom extirpated and therefore experience fewer fitness consequences by living alone (Chapter 3; Wcislo 1987). Thirdly, stem nesting bees are generally less sensitive to harsh ecological conditions than ground nesting bees. By nesting in elevated mirco-environments prolonged periods of rain do not lead to brood rot or the need for nest reconstruction (Chapter

3) as seen in ground nesting bees whose subterranean tunnels are often destroyed and waterlogged under such conditions (Packer et al. 1989b; Packer 1992; Richards and Packer 1995).

Behavioural Factors Contributing to Sociality

Female biased sex allocation has been linked to sociality in many social insects (Trivers and Hare 1976; Seger 1983; Schwarz 1988). *Ceratina* species are no exception to this finding in that female biased numerical sex ratios are associated with facultative sociality in the Old World *Ceratina* species studied to date (Chapter 5; Table 1). Furthermore, all species studied to date are sexually dimorphic with females typically larger than males indicating female-biased numerical sex ratios and female-biased investment ratios in many species. Conversely, studies on North American species have shown equal investment patterns in the solitary species. These species produce male-biased numerical sex ratios in balance to the female biased cost ratio.

In addition to female-biased sex allocation and investment in the Old World, some species are reported to form multifemale nests (Chapters 2 and 5). Before this thesis, the best studied examples were *Ceratina japonica* (30%), *C. okinawana* (20%), and *C. flavipes* (<1%) from Japan (percent multifemale brood rearing nests in the wild). During a 1958-59 survey of Australia Michener only anecdotally described finding a single multifemale *C. australensis* nest with brood (Michener 1962). However, I have shown this population is comparable to Japanese congeners in that approximately 13% of all brood rearing colonies contain two cohabiting adult females (Chapters 2 and 3; Rehan et al. 2010). Contrary to Old World findings, North American species have consistently male-biased sex allocation and social colonies have never been found (Kislow 1976; Johnson 1988; Rehan and Richards 2010).

The formation of multifemale colonies is not only associated with female-biased sex allocation but also corresponds to patterns of nest reuse. In Old World species, females are reported to reuse nests (Chapters 2, 3 and 5; Table 1) and the greater the frequency of nest reuse in the wild the greater the frequency of social colonies. Conversely, North American species which always disperse to find new nesting substrates each spring have never been recorded to form social colonies. These data reveal that philopatry, or remaining at the natal nest greatly increases the probability of social colony formation. Social colonies are rarely established in newly initiated nests suggesting that cohabitation is a result of philopatry and also that co-nesting females are likely kin. In Chapter 4, I present the first direct study of genetic relatedness in the Ceratinini. Here I found that for *C. australensis* social pairs consisted of full sisters that remain at the natal nest after adult eclosion for an additional one to two subsequent brood rearing seasons. Despite no other genetic data at present to confirm the nature of multifemale associations in other Old World congeners, it seems probable that in these species social colonies in reused nests also form from closely related sororal or matrifilial kin groups.

Reproductive Division of Labour

Once multifemale associations evolve a reproductive division of labour between females follows. There are no reported instances of egalitarian or communal breeding in *Ceratina*. Social females divide reproduction and foraging tasks. In *C. australensis,* co-nesting females are of equivalent body size and age class and are full sisters (Chapter 4). In social pairs, the primary female is dominant in reproduction and foraging behaviours while the secondary female remains at the nest as a passive guard, contributing no eggs or pollen to the nest (Chapter 2). It remains unknown how reproductive division of labour is decided, but if the primary female eclosed hours to days earlier than the secondary female this might be

adequate to bequeath a dominance hierarchy between nestmates, as found in some allodapine bees (Schwarz and O'Keefe 1991). In *C. australensis* and many allodapine bees, secondary females remain at the natal nest in waiting for nest inheritance. Once the dominant dies the secondary becomes both reproductively active and commences foraging activity for their own brood (Chapter 4).

More pronounced and perhaps easier to explain is the reproductive division of labour among Japanese ceratinines. In these species body size varies considerably among females and reproductive skew follows size and age-based dominance hierarchies. Larger and older females typically become primary reproductives and guard the nest while smaller and younger females act as non-reproductive foragers (Sakagami and Maeta 1987, 1989, 1995; Hogendoorn and Velthuis 1999). This behaviour is hierarchical in which reproductive females do not take on risky foraging activity and instead remain at the nest and develop their ovaries and lay eggs on the secondary female's pollen provisions. Secondary workers in these species are born of maternal manipulation as mothers under-provision innermost brood cells to make dwarf eldest daughters. These females are first to eclose in their natal nest and can act as foragers that feed siblings prior to overwintering if the mother dies, and perhaps even if the mother is still alive (Sakagami and Maeta 1977).

Origins and Diversity

In Chapter 6 (Rehan et al. 2010), I present the first robust molecular phylogeny on the origin of the Ceratinini in combination with fossil dating from amber preservations (Engel 2001). Earlier cladistic work suggested an African or an Asian origin of this tribe, but former analyses lacked calibration points or outgroup reference points (Terzo 2000). My molecular phylogeny and historical biogeography of the ceratinines revealed an African origin with Old World radiation following a New World invasion (Rehan et al. 2010). Ceratinini are a truly

cosmopolitan tribe found on every continent except Antarctica, with great diversity and speciation on all continents but Australia home to a single species, *Ceratina (Neoceratina) australensis* (Michener 1962). Despite their distribution and abundance no further phylogenetic or biogeographic studies have been conducted on this tribe.

Evolutionary Considerations

Although studies of the social behaviour of the Ceratinini are in their infancy, underlying patterns can be viewed across the tribe. The assessment of current behaviours and adaptive values for specific traits does not necessarily equate to the historical processes producing each phenotype. Selective forces producing social phenotypes might be quite different than those maintaining sociality. Therefore, future work on the phylogenetic or evolutionary context is imperative to trace the maintenance and elaboration versus origins and losses of social behaviour over time.

Taken together we see that social nesting is recurrent in Old World species and not observed in North American studies. It should be noted that sociality is always a low frequency phenomenon when it occurs, at best representing a third of the population. The fact that the majority of colonies remain solitary indicates that solitary nesting is not maladaptive in the studied species. This suggests rather that sociality, although quite common among Old World species, provides no resounding advantage and thus does not spread to fixation as a more obligate social phenotype. Perhaps harsher selective environments as found in desert or tundra environments might necessitate obligate social colony formation. Perhaps *Ceratina* are simply a species fully capable of forming social colonies but not experiencing the strict selective regimes required for sociality to evolve in the first place.

Experiments placing normally solitary species into artificial social groups suggest that social trademarks such as mutual tolerance and reproductive division of labour may be an emergent property of incipient social groups rather than a subsequent adaptation after groups were formed (Sakagami and Maeta 1987; Fewell and Page 1999; Helms Cahan and Fewell 2004). In addition to mutual tolerance and prolonged cohabitation, subsociality is quite frequent in many organisms yet further elaboration into eusocial life clearly requires very specific selective environments that are rare in nature. The ceratinines are quite capable of forming eusocial colonies as this behaviour is observed naturally in some species and can be provoked in others. Although eusociality has led to the great ecological success of some lineages, for others, including the small carpenter bees, social organization has disadvantages preventing further elaboration of this trait.

Taken together, *Ceratina* are a diverse and labile model system to uncover the ecological and genetic origins and elaborations of sociality. Future work is needed to determine the social behaviour across this group. Studies to date suggest life history traits including philopatry, mutual tolerance and overlapping generations largely facilitate sociality in the ceratinines. Conversely, dispersal, antagonism and discrete generations impede further social behaviour in some lineages. With a comprehensive phylogeny set in place now is the time to elaborate on this work to include the life history and social potential of more species to resolve the origin and diversification of the small carpenter bees.

Table 1: Some life history traits of *Ceratina* species, their geographic distributions and demographic data. *Location* is the study region; *Voltinism* is the number of reproductive broods per year: uni = one, bi = two, and multi = >2; *Sociality* is the social potential of each species in the wild: solitary = never observed forming multiple female brood rearing colonies and social = multiple adult female brood rearing colonies observed; *sex ratio* reported as proportion of males; *body size ratio* reported as body size of females/body size of males; *nest reuse* = frequency of twig reuse for a second reproductive brood; *MFN* = frequency of multiple female colonies in the wild; ? = unknown

Subgenus	Species	Location	Voltinism	Sociality	Sex ratio	Body size ratio	Nest reuse	MFN	Reference
Zadontomerus	*calcarata*	N. America	uni	Solitary	0.57	1.31	0	0	Rehan & Richards 2010
Zadontomerus	*dupla*	N. America	uni	Solitary	0.55	1.55	0	0	Grothaus 1962
Zadontomerus	*strenua*	N. America	uni	Solitary	0.63	?	0	0	Kislow 1976
Ceratinidia	*japonica*	Asia	uni	Social	0.23	?	0.35	0.20	Sakagami & Maeta 1977
Ceratinidia	*okinawana*	Asia	multi	Social	?	?	0.21	0.11	- & - 1989, 1995
Ceratinidia	*flavipes*	Asia	uni	Social	0.48	1.46	?	0.01	- & - 1987
Ceratina	*megastigmata*	Asia	uni	Social	0.41	?	0.31	0.05	Katayama & Maeta 1979
Ceratina	*iwatai*	Asia	bi	Social	?	?	0.55	0.55	Maeta 1993
Neoceratina	*australensis*	Australia	bi	Social	0.35	1.09	0.33	0.13	Rehan unpub. data
Neoceratina	*dentipes*	Asia	multi	Social	0.17	?	0.08	0.08	Rehan et al. 2009
Pithitis	*smaragdula*	Asia	multi	Social	?	1.11	?	0.20	Rehan et al. 2009
Ceratinidia	*accusator*	Asia	multi	Social	0.11	1.14	0	?	Rehan et al. 2009
Ceratinidia	*nigrolateralis*	Asia	multi	Social	0.19	1.01	0.14	0.10	Rehan et al. 2009
Euceratina	*dallatorreana*	N. America	bi	Solitary	0	?	0	0	Daly 1966
Ctenoceratina	*moerenhouti*	Africa	?	Social	?	?	?	?	Daly 1988
Ceratina	*subquadrata*	Africa	?	Social	0.03	1.19	?	0.08	Rehan unpub. data

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